PB84-220359

Health Assessment Document for Chlorinated Benzenes. Parts 1 and 2 External Review Draft

(U.S.) Environmental Protection Agency
Cincinnati, OH

Apr 84

U.S. Department of Commerce
National Technical Information Service
NTIS

United States
Environmental Protection
Agency

Office of Health and
Environmental Assessment
Washington DC 20460

EPA-600/8-84-015A
April 1984
External Review Draft

Research and Development

PB84-220359

EPA

Health Assessment Document for Chlorinated Benzenes

Part 1 of 2

Review
Draft

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TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/8-84-015A
2.
3. RECIPIENT'S ACCESSION NO. PB84 220359
4. TITLE AND SUBTITLE

HEALTH ASSESSMENT DOCUMENT FOR CHLORINATED BENZENES
Review Draft

5. REPORT DATE April 1984
6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)
8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS

same as #12

10. PROGRAM ELEMENT NO.
11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS

Environmental Criteria and Assessment Office
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

13. TYPE OF REPORT AND PERIOD COVERED
14. SPONSORING AGENCY CODE EPA/600/22
15. SUPPLEMENTARY NOTES
16. ABSTRACT

This document summarizes the current knowledge of the effects of exposure to the chlorinated benzenes on human health.

The chlorinated benzenes are resistant to chemical and biological degradation and tend to accumulate in lipid-containing tissues of animals and humans. The ubiquitous environmental distribution of the chlorinated benzenes and their bioconcentration in humans are a basis for concern over the consequences of chronic exposure to human health.

The rationale for structuring this document is based primarily on two major issues, exposure and response. The first portion of the document is devoted to the chlorinated benzenes in the environment: physical and chemical properties, the monitoring of the chlorinated benzenes in various media, natural and human-made sources, the transport and distribution of the chlorinated benzenes within environmental media, and the levels of exposure. The second part is devoted to biological responses in laboratory animals and humans including metabolism, pharmacokinetics, as well as the toxicological effects of the chlorinated benzenes.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT
19. SECURITY CLASS *(This Report)*
20. SECURITY CLASS *(This page)*
21. NO. OF PAGES 632
22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

EPA-600/8-84-015A
April 1984
External Review Draft

DRAFT
Do not cite or quote

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DISCLAIMER

This report is an external draft for review purposes only and does not constitute Agency policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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PREFACE

The Office of Health and Environmental Assessment of the Office of Research and Development has prepared this Health Assessment Document (HAD) on chlorinated benzenes at the request of the Office of Air Quality, Planning and Standards. The chlorinated benzenes are a group of 12 chlorinated cyclic aromatic compounds which are currently being studied by the Environmental Protection Agency (EPA) to determine if they should be regulated as hazardous air pollutants under the Clean Air Act.

In the development of this assessment document, the scientific literature has been searched and inventoried, key studies have been reviewed and evaluated and summaries and conclusions have been directed at identifying the health effects from exposure to the various chlorinated benzenes. At several stages in the HAD development process, the chlorinated benzenes document has been reviewed for scientific and technical accuracy. These peer reviews have been by scientists from inside and outside the EPA. Observed effect levels and dose-response relationships are discussed where appropriate in order to identify the critical effect and to place adverse health responses in perspective with observed environmental levels.

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TABLE OF CONTENTS

Page

1. INTRODUCTION. 1-1

2. SUMMARY AND CONCLUSIONS 2-1

  2.1. SUMMARY 2-1

    2.1.1. Properties, Production and Use. 2-1
    2.1.2. Environmental Levels, Transport and Fate. 2-2
    2.1.3. Ecological Effects. 2-4
    2.1.4. Pharmacokinetics. 2-5
    2.1.5. Effects on Humans 2-10
    2.1.6. Mammalian Toxicology. 2-12

  2.2. CONCLUSIONS 2-20
  2.3. NEEDS FOR FUTURE RESEARCH 2-20

3. PHYSICAL AND CHEMICAL PROPERTIES/ANALYTICAL METHODOLOGY 3-1

  3.1. SYNONYMS, TRADE NAMES AND IDENTIFICATION NUMBERS. 3-1
  3.2. PHYSICAL AND CHEMICAL PROPERTIES. 3-1
  3.3. ANALYTICAL METHODOLOGY. 3-15

    3.3.1. Chemical Analysis in Air. 3-15
    3.3.2. Chemical Analysis in Water. 3-18
    3.3.3. Chemical Analysis in Soil, Sediment and Chemical Waste Disposal Site Samples 3-19
    3.3.4. Chemical Analysis in Fish and Other Foods 3-20

  3.4. SUMMARY 3-23

4. PRODUCTION, USE AND ENVIRONMENTAL LEVELS. 4-1

  4.1. PRODUCTION. 4-1
  4.2. USE 4-5
  4.3. SOURCE AND ENVIRONMENTAL LEVELS 4-5

    4.3.1. Levels in Air 4-12
    4.3.2. Water 4-18
    4.3.3. Food. 4-26
    4.3.4. Soil and Sediments. 4-28
    4.3.5. Human Tissue Residues 4-29

  4.4. RELATIVE SOURCE CONTRIBUTIONS TO TOTAL EXPOSURE 4-34

    4.4.1. Air 4-36
    4.4.2. Water 4-36
    4.4.3. Food. 4-38

  4.5. SUMMARY 4-38

Page

5. ENVIRONMENTAL TRANSPORT AND FATE. 5-1
  5.1. TRANSPORT 5-1
    5.1.1. Air 5-1
    5.1.2. Water 5-2
    5.1.3. Soil. 5-4
  5.2. FATE. 5-7
    5.2.1. Air 5-7
    5.2.2. Water 5-8
    5.2.3. Soil. 5-10
  5.3. BIOCONCENTRATION, BIOACCUMULATION AND BIOMAGNIFICATION. . . 5-13
  5.4. SUMMARY 5-19
6. ECOLOGICAL EFFECTS. 6-1
  6.1. EFFECTS ON THE AQUATIC ENVIRONMENT. 6-1
    6.1.1. Effect on Freshwater and Marine Fish. 6-1
    6.1.2. Effect on Aquatic Crustaceans 6-14
    6.1.3. Embryotoxic and Reproductive Effects. 6-16
    6.1.4. Effect on Aquatic Plants. 6-24
    6.1.5. Residues. 6-30
  6.2. EFFECTS ON NONAQUATIC ENVIRONMENTS. 6-34
    6.2.1. Plants. 6-34
    6.2.2. Insects 6-35
    6.2.3. Birds 6-37
    6.2.4. Residues. 6-38
  6.3. SUMMARY 6-39
7. MONOCHLOROBENZENE 7-1
  7.1. PHARMACOKINETICS. 7-1
    7.1.1. Absorption. 7-1
    7.1.2. Distribution. 7-1
    7.1.3. Metabolism. 7-2
    7.1.4. Excretion 7-5
    7.1.5. Summary 7-10
  7.2. EFFECTS ON HUMANS 7-10

Page

7.3. MAMMALIAN TOXICITY. 7-12

7.3.1. Acute Toxicity. 7-12
7.3.2. Subchronic Toxicity 7-15
7.3.3. Chronic Toxicity. 7-23
7.3.4. Mutagenicity. 7-24
7.3.5. Carcinogenicity 7-24
7.3.6. Reproductive and Teratogenic Toxicity 7-31

7.4. INTERACTIONS. 7-31
7.5. SUMMARY 7-32

8. DICHLOROBENZENES. 8-1

8.1. PHARMACOKINETICS. 8-1

8.1.1. Absorption. 8-1
8.1.2. Distribution. 8-3
8.1.3. Metabolism. 8-6
8.1.4. Excretion 8-8
8.1.5. Summary 8-9

8.2. EFFECTS ON HUMANS 8-10

8.2.1. Occupational Studies. 8-10
8.2.2. Case Studies. 8-11
8.2.3. Summary 8-17

8.3. MAMMALIAN TOXICOLOGY. 8-17

8.3.1. Acute Toxicity. 8-17
8.3.2. Subchronic Toxicity 8-22
8.3.3. Chronic Toxicity. 8-32
8.3.4. Mutagenicity. 8-33
8.3.5. Carcinogenicity 8-34
8.3.6. Reproductive and Teratogenic Toxicity 8-39

8.4. INTERACTIONS. 8-39
8.5. SUMMARY 8-40

9. TRICHLOROBENZENES 9-1

9.1. PHARMACOKINETICS. 9-1

9.1.1. Absorption. 9-1
9.1.2. Distribution. 9-2
9.1.3. Metabolism. 9-2
9.1.4. Excretion 9-6
9.1.5. Summary 9-8

9.2. EFFECTS IN HUMANS 9-9

Page

9.3. MAMMALIAN TOXICOLOGY 9-9

9.3.1. Acute Toxicity 9-9
9.3.2. Subchronic Toxicity 9-14
9.3.3. Chronic Toxicity 9-23
9.3.4. Mutagenicity 9-24
9.3.5. Carcinogenicity 9-25
9.3.6. Reproductive and Teratogenic Toxicity 9-26

9.4. INTERACTIONS 9-28
9.5. SUMMARY 9-28

10. TETRACHLOROBENZENES 10-1

10.1. PHARMACOKINETICS 10-1

10.1.1. Absorption 10-1
10.1.2. Distribution 10-2
10.1.3. Metabolism 10-7
10.1.4. Excretion 10-10
10.1.5. Summary 10-13

10.2. EFFECTS ON HUMANS 10-14
10.3. MAMMALIAN TOXICOLOGY 10-15

10.3.1. Acute Toxicity 10-15
10.3.2. Subchronic Toxicity 10-20
10.3.3. Chronic Toxicity 10-22
10.3.4. Mutagenicity 10-22
10.3.5. Carcinogenicity 10-23
10.3.6. Reproductive and Teratogenic Effects 10-23

10.4. INTERACTIONS 10-24
10.5. SUMMARY 10-24

11. PENTACHLOROBENZENE 11-1

11.1. PHARMACOKINETICS 11-1

11.1.1. Absorption 11-1
11.1.2. Distribution 11-2
11.1.3. Metabolism 11-7
11.1.4. Excretion 11-11
11.1.5. Summary 11-13

11.2. EFFECTS ON HUMANS 11-13

Page

11.3. MAMMALIAN TOXICOLOGY. 11-13

11.3.1. Acute Toxicity. 11-13
11.3.2. Subchronic Toxicity 11-16
11.3.3. Chronic Toxicity. 11-18
11.3.4. Mutagenicity. 11-19
11.3.5. Carcinogenicity 11-19
11.3.6. Reproductive and Teratogenic Toxicity 11-19

11.4. INTERACTIONS. 11-26
11.5. SUMMARY 11-26

12. HEXACHLOROBENZENE 12-1

12.1. PHARMACOKINETICS. 12-1

12.1.1. Absorption. 12-1
12.1.2. Distribution. 12-3
12.1.3. Metabolism. 12-16
12.1.4. Excretion 12-20
12.1.5. Summary 12-27

12.2. EFFECTS ON HUMANS 12-29

12.2.1. Epidemiologic Studies 12-29
12.2.2. Accidental Ingestion in Turkey. 12-32
12.2.3. Summary 12-37

12.3 MAMMALIAN TOXICOLOGY. 12-40

12.3.1. Acute Toxicity. 12-40
12.3.2. Subchronic Toxicity 12-42
12.3.3. Chronic Toxicity. 12-56
12.3.4. Mutagenicity. 12-59
12.3.5. Carcinogenicity 12-60
12.3.6. Reproductive and Teratogenic Effects. 12-125

12.4. INTERACTIONS. 12-131
12.5. SUMMARY 12-134

13. OVERVIEW OF EFFECTS OF MAJOR CONCERN. 13-1

13.1. PRINCIPAL EFFECTS AND TARGET ORGANS 13-1
13.2. ANIMAL TOXICITY STUDIES MOST USEFUL FOR HEALTH ASSESSMENT AND ESTIMATED TOXICITY THRESHOLDS. 13-5

13.2.1. Animal Toxicity Studies 13-5
13.2.2. Estimated Toxicity Thresholds 13-31

13.3. CARCINOGENICITY STUDIES 13-31
13.4. HUMAN STUDIES 13-39

Page

13.5. FACTORS INFLUENCING HEALTH HAZARD ASSESSMENT. 13-40

13.5.1. Exposure. 13-40

13.6. REGULATIONS AND STANDARDS 13-45

13.6.1. Occupational Standards. 13-45
13.6.2. Transportation Regulations. 13-51
13.6.3. Solid Waste Regulations 13-52
13.6.4. Food Tolerances 13-54
13.6.5. Water Regulations 13-54
13.6.6. Air Regulations 13-55

14. REFERENCES. 14-1

APPENDIX A: Comparison Among Different Extrapolation Models. A-1

LIST OF TABLES

No.	Title	Page
3-1	Synonyms, Trade Names and Identification Numbers of the Chlorinated Benzenes.	3-3
3-2	Physical Properties of the Chlorinated Benzenes	3-7
3-3	Vapor Pressures and Vapor Densities of the Chlorinated Benzenes.	3-8
3-4	Reported Composition of Commercial 1,2-Dichlorobenzene.	3-11
4-1	United States Production of Chlorinated Benzenes for Selected Years.	4-2
4-2	U.S. Producers and Estimated Annual Production Capacities (1983) of Chlorobenzenes.	4-6
4-3	A Summary of the Uses of the Chlorinated Benzenes	4-8
4-4	Estimated Quantities of Chlorobenzenes Lost During Manufacture, and to the Environment Compared with Total Production in 1983.	4-9
4-5	Estimated Quantities of Hexachlorobenzene (HCB) in Industrial Wastes and Byproducts in 1972.	4-11
4-6	Chlorinated Benzene Levels in Ambient Air from Different Locations in the U.S.	4-13
4-7	Concentrations of Chlorinated Benzenes at Three Sites	4-16
4-8	Overall and Site-Specific Mean Atmospheric Levels of Chlorobenzenes throughout the United States	4-17
4-9	Atmospheric Levels of Hexachlorobenzene Around Selected Industrial Plants	4-19
4-10	Chlorinated Benzenes in Surface Water	4-20
4-11	Chlorobenzene Concentrations in Drinking Water from Ontario, Canada	4-24
4-12	Frequency and Range of Concentrations of Chlorinated Benzenes Pollutants in Industrial Wastewaters	4-27
4-13	Chlorinated Benzene Residues in Human Adipose Tissue.	4-30
4-14	Chlorinated Benzenes in the Blood of Nine Residents of Love Canal in Niagara Falls, New York	4-33

No.	Title	Page
4-15	Chlorinated Benzenes in the Breath and Urine of Nine Residents of Love Canal in Niagara Falls, New York.	4-35
4-16	Estimated Yearly Exposure to Several Chlorinated Benzenes Via Inhalation.	4-37
5-1	Predicted Transport and Fate of Chlorinated Benzenes Released from Landfills and Lagoons	5-5
5-2	Transport of Chlorinated Benzenes in Sandy Soil	5-6
5-3	Estimated Atmospheric Residence Time and Daily Loss Rates for Several Chlorinated Benzenes.	5-9
5-4	Aqueous Biodegradability Studies of Chlorinated Benzenes. . .	5-11
5-5	Octanol/Water Partition Coefficients, Bioconcentration Factors and Biological Half-lives for Chlorinated Benzenes in Fish	5-15
5-6	Bioconcentration Factor and Slope of the Elimination Curve for Guppies (Poecilia reticulata) Exposed to Six Chlorinated Benzenes.	5-18
6-1	Acute Toxicity Data for Fish Species Exposed to Chlorinated Benzenes.	6-[illegible]
6-2	Chronic Toxicity Values of Chlorinated Benzenes in Fish . . .	6-12
6-3	Bioconcentration Factors of Some Chlorinated Benzenes in Two Fish Species	6-15
6-4	Acute Toxicity Data for Crustaceans Exposed to Chlorinated Benzenes.	6-17
6-5	Embryo-Larval Toxicity of Monochlorobenzene to Goldfish, Largemouth Bass and Rainbow Trout in Soft and Hard Water. . .	6-21
6-6	Results of 1,2,4,5-Tetrachlorobenzene Tests with Embryo to Juvenile Sheepshead Minnows in Continuous-Flow Natural Seawater.	6-23
6-7	Adult Lifespan and Reproductive Performance of Brine Shrimp Exposed to 1,3,5-Trichlorobenzene.	6-25
6-8	Acute Toxicity Data for Aquatic Algae Exposed to Chlorinated Benzenes.	6-26
6-9	Chlorinated Benzene Concentrations ($\mu g/\ell$) in Water and Sediment.	6-31

No.	Title	Page
6-10	Chlorinated Benzene Concentrations in a Variety of Marine Species	6-32
6-11	Emergence of Adult Houseflies 8 Days Following Exposure of Pupae to "Saturation Concentration" of Dichlorobenzene Vapors	6-36
6-12	Chlorinated Benzene Residues in Bird Eggs	6-40
7-1	Percentage of Isomers of Chlorophenol from Metabolism of Monochlorobenzene	7-4
7-2	Species Variation in Urinary Metabolites of ^{14}C-Monochlorobenzene	7-9
7-3	Acute Toxicity of Monochlorobenzene	7-14
7-4	Summary of Subchronic Toxicity Studies on Monochlorobenzene	7-16
7-5	Mutagenicity Testing of Monochlorobenzene	7-25
7-6	Nonneoplastic Lesions in F344 Rats Given Chlorobenzene by Gavage for 2 Years	7-28
7-7	Statistical Comparisons of Liver Tumors in Male Rats Treated with Chlorobenzene and Vehicle Controls	7-29
8-1	Tissue Concentrations of 1,4-Dichlorobenzene in Adult Female CFY Rats	8-5
8-2	Chromosomal Alterations in Persons Accidentally Exposed to 1,2-Dichlorobenzene	8-12
8-3	Case Reports Involving Dichlorobenzenes (DCB)	8-13
8-4	Acute Toxicity of 1,2-Dichlorobenzene	8-19
8-5	Acute Toxicity of 1,4-Dichlorobenzene	8-20
8-6	Subchronic Toxicity of 1,2-Dichlorobenzene	8-23
8-7	Subchronic Toxicity of 1,4-Dichlorobenzene	8-25
8-8	NTP Bioassay of 1,2-Dichlorobenzene Analysis of Primary Tumors in Male Rats: Adrenal Pheochromocytomas	8-36
9-1	Distribution of ^{14}C-Labeled 1,2,4-Trichlorobenzene in Rat Tissues after Oral Dosing with 181.5 mg/kg/day for 7 Days	9-3
9-2	Summary of Subchronic and Chronic Toxicity Studies on Trichlorobenzenes	9-15

No.	Title	Page
10-1	Percentage of 1,2,4,5-Tetrachlorobenzene Steady-State Reached at Specific Times in Fat and Plasma of Dogs	10-4
10-2	Time Required to Reach Various Percentages of 1,2,4,5-Tetrachlorobenzene Steady-State in Fat and Plasma of Dogs	10-5
10-3	Unchanged Tetrachlorobenzene in Rabbit Tissues 6 Days After Oral Dosing	10-6
10-4	Urinary Metabolites of Tetrachlorobenzene Isomers in Rabbits 6 Days After Oral Dosing	10-9
10-5	Summary of Excretion of the Isomeric Tetrachlorobenzenes as Metabolites or as Unchanged Compound in Rabbits Dosed Orally	10-11
10-6	Excretion of Unchanged Tetrachlorobenzenes in the Expired Air of Rabbits After Oral Dosing	10-12
10-7	Frequency of Chromatid-type Chromosome Aberrations in Peripheral Lymphocytes	10-16
10-8	Frequency of Labile Chromosome-type Aberrations	10-17
10-9	Frequency of Stable Chromosome-type Aberrations	10-18
10-10	Summary of Toxicity Studies on Tetrachlorobenzenes	10-19
11-1	Distribution of Pentachlorobenzene Residues in the Tissues of Maternal Rats after Oral Administration	11-3
11-2	Distribution of Pentachlorobenzene Residues in the Tissues of Fetal Rats after Oral Administration to Dams	11-4
11-3	Distribution of Pentachlorobenzene and/or Metabolites on the 40th Day in the Rhesus Monkey Following a Single Oral Dose of 0.5 mg/kg Body Weight	11-6
11-4	Distribution of Pentachlorobenzene in Chinchilla Doe Rabbits Expressed as a Percentage of Administered Dose	11-8
11-5	Percentage of Pentachlorobenzene and Its Metabolites Identified in Urine, Feces and Various Organs of Rhesus Monkeys Dosed 0.5 mg/kg Body Weight Pentachlorobenzene	11-9
11-6	Cumulative Urinary and Fecal Excretion of Pentachlorobenzene and Metabolites During 40 Days Following a Single Oral Dose of 0.5 mg/kg in Male and Female Rhesus Monkeys	11-12
11-7	Acute Oral Toxicity of Pentachlorobenzene	11-15

No.	Title	Page
11-8	Summary of Subchronic, Reproductive and Teratogenic Toxicity Studies on Pentachlorobenzene.	11-17
11-9	Reproductive Effects in Litters of Female Rats Fed Diets Containing Pentachlorobenzene	11-21
11-10	Toxic Effects of Pentachlorobenzene on Reproduction in Rats Dosed on Each of Gestation Days 6-15	11-23
11-11	Skeletal and Soft-Tissue Abnormalities Observed in Rat Litters of Dams Treated with Pentachlorobenzene on Each of Gestation Days 6-15.	11-24
11-12	Fetal Wistar Rat Residues of Pentachlorobenzene	11-25
12-1	Storage and Excretion of ^{14}C-HCB Administered Orally in Arachis Oil in Rats.	12-4
12-2	Tissue Concentration (ppm) of ^{14}C-Hexachlorobenzene and Its Metabolites in Sprague-Dawley Rats.	12-6
12-3	Tissue Levels of HCB (ppm) in Adult Female Rhesus Monkeys . .	12-7
12-4	HCB Concentrations in Tissues of Male Beagles Receiving Single Intravenous Doses of 1 mg/kg bw in Olive Oil	12-9
12-5	Mean (±SE) Hexachlorobenzene Radioactivity (dpm/g) of Selected European Ferret Tissues.	12-14
12-6	Mean (±SE) HCB Radioactivity (dpm x 10^3) of European Ferret Kits	12-15
12-7	Concentrations of HCB and its Metabolites (mg/kg) in the Liver and Kidneys of Male and Female Rats	12-19
12-8	Hexachlorobenzene and Its Major Metabolites in the Excreta of Different Animal Species	12-21
12-9	Results of Blood and Urine Analysis in Men Employed in a Chlorinated Solvents Plant, 1974-1977	12-31
12-10	HCB Plasma Levels in Exposed Individuals and Controls	12-33
12-11	Clinical Signs and Symptoms in Humans 25 Years After Exposure to Low Levels in HCB in Turkey, 1955-1959.	12-36
12-12	Porphyrin Levels in Patients and Controls	12-38
12-13	Laboratory Test Results of Turkish Patients	12-39
12-14	Summary of Toxicity Studies on Hexachlorobenzene.	12-43

No.	Title	Page
12-15	Porphyrin Content and Uroporphyrinogen Decarboxylase Activity in the Liver Cytosol of Female Rats Pretreated with 100 mg/kg HCB Every Other Day for 6 Weeks.	12-53
12-16	Tumor Incidence in Hamsters Given HCB in the Diet	12-62
12-17	HCB Levels in Tissues of Male Rats Following Administration of 8 mg/kg in Sunflower Oil for 19 Days	12-64
12-18	HCB Levels in Tissues of Male Rats Following Administration of ^{14}C-HCB in Arachis Oil	12-65
12-19	Effect of HCB on Hamsters: Liver Tumors and Other Liver Lesions	12-69
12-20	Liver Tumor Incidence in Mice Fed HCB	12-71
12-21	Tumor Data on Mice Fed HCB.	12-72
12-22	Body Weights of Female Agus Rats Fed Hexachlorobenzene for 90 Weeks.	12-76
12-23	Growth Rates for Female Agus Rats on a Diet Containing 100 ppm HCB	12-78
12-24	Dosage Levels in the Chronic Feeding Study of Hexachlorobenzene in Sprague-Dawley Rats.	12-81
12-25	Liver and Kidney Tumors in Sprague-Dawley Rats Given Hexachlorobenzene in the Diet for up to 2 Years	12-82
12-26	Adrenal Tumors in Sprague-Dawley Rats Given Hexachlorobenzene in the Diet for up to 2 Years	12-84
12-27	Exposure Levels in the Chronic Feeding, 2-Generation Study of Hexachlorobenzene in Sprague-Dawley Rats	12-86
12-28	Tumors in Organs that Showed Statistical Differences from Control in F_1 Sprague-Dawley Rats Treated with Hexachlorobenzene	12-88
12-29	Parathyroid and Adrenal Pheochromocytomas in Sprague-Dawley Rats Maintained on Synthetic Diets of Varying Vitamin A Content and With or Without Hexachlorobenzene . . .	12-89
12-30	Qualitative Comparison of Tumor Development in Rats Following Hexachlorobenzene Administration in Different Studies	12-92
12-31	Tumor Incidences in Male and Female Hamsters Given Hexachlorobenzene in Diet	12-107

No.	Title	Page
12-32	Incidence of Liver Cell Tumors in Male and Female Swiss Mice Given Hexachlorobenzene Diet	12-108
12-33	Liver and Kidney Tumor Incidence Rates in Male and Female Sprague-Dawley Rats Given Hexachlorobenzene in Diet	12-109
12-34	Incidence Rate of Adrenal Pheochromocytoma in Female Sprague-Dawley Rats (F_1 generation) in a 2-Generation Feeding Study	12-110
12-35	The Carcinogenic Potency of Hexachlorobenzene, Calculated on the Basis of 14 Data Sets, Using the Linearized Multistage Model	12-113
12-36	Upper-Bound (Point) Estimation of Risk, Based on Hepatocellular Carcinoma in Female Rats	12-115
12-37	Relative Carcinogenic Potencies Among 54 Chemicals Evaluated by the Carcinogen Assessment Group as Suspect Human Carcinogens	12-119
12-38	Significantly Increased Incidence of Tumors in Animals Given Hexachlorobenzene in Diet	12-124
12-39	Analysis of the Excreta from Rats Administered Hexachlorobenzene After an Initial Treatment with Diethylstilboestrol	12-132
13-1	Summary of Subchronic Toxicity Studies on Monochlorobenzene	13-6
13-2	Subchronic Toxicity of 1,2-Dichlorobenzene	13-9
13-3	Subchronic Toxicity of 1,4-Dichlorobenzene	13-11
13-4	Summary of Subchronic and Chronic Toxicity Studies on Trichlorobenzenes	13-13
13-5	Summary of Toxicity Studies on Tetrachlorobenzenes	13-15
13-6	Summary of Subchronic, Reproductive and Teratogenic Toxicity Studies on Pentachlorobenzene	13-16
13-7	Summary of Toxicity Studies on Hexachlorobenzene	13-17
13-8	Comparison of Toxic Effects of Chlorinated Benzene Isomers in Rats	13-22
13-9	Comparison of Toxic Effects of Chlorinated Benzene Isomers in Mice	13-24
13-10	Comparison of Toxic Effects of Chlorinated Benzene Isomers in Rabbits	13-26

No.	Title	Page
13-11	Comparison of Toxic Effects of Chlorinated Benzene Isomers in Dogs	13-28
13-12	Comparison of Toxic Effects of Chlorinated Benzene Isomers in Monkeys	13-30
13-13	Toxicity Data for Threshold Estimates	13-32
13-14	Summary of Tumors Induced in Rodents by HCB	13-37
13-15	Comparison of Chemical and Physical Properties of Chlorinated Benzenes	13-41
13-16	Comparison of Chlorinated Benzenes BCF and Water Concentrations	13-42
13-17	Estimated Yearly Exposure to Several Chlorinated Benzenes Via Inhalation	13-44
13-18	Occupational Standards for Monochlorobenzene	13-46
13-19	Occupational Standards for 1,2-Dichlorobenzene	13-48
13-20	Occupational Standards for 1,4-Dichlorobenzene	13-49
13-21	The Chlorinated Benzenes as Constituents of Hazardous Wastes from Specific Sources	13-53
13-22	Ambient Water Quality Criteria for Chlorinated Benzenes--Aquatic Life	13-56
13-23	Ambient Water Quality Criteria for the Chlorinated Benzenes for the Protection of Human Health	13-57
13-24	Maximum Immission Concentration Standards for Monochlorobenzene	13-59

LIST OF FIGURES

No.	Title	Page
3-1	Chemical Structure of the Chlorinated Benzenes	3-2
7-1	Metabolism of Monochlorobenzene	7-7
9-1	Metabolic Pathways for Trichlorobenzene (TCB) Isomers Through Arene Oxide Intermediates in Rabbits	9-7
12-1	Histogram Representing the Frequency Distribution of the Potency Indices of 54 Suspect Carcinogens Evaluated by the Carcinogen Assessment Group	12-118

1. INTRODUCTION

The purpose of this document is to summarize the current knowledge of the effects of exposure to the chlorinated benzenes on human health.

The chlorinated benzenes are a group of 12 compounds in which 1 to 6 chlorine atoms have been substituted for the hydrogens on a benzene ring. They are used as chemical intermediates in the synthesis of pesticides and other chlorinated compounds, and as solvents, pesticides, dye carriers, space deodorants and other products. Environmental contamination results from emissions to air and water during the manufacture and use of the chlorinated benzenes and from the disposal of wastes from a number of processes. These compounds are resistant to chemical and biological degradation and tend to accumulate in lipid-containing tissues of animals and humans. The ubiquitous environmental distribution of the chlorinated benzenes and their bioconcentration in humans are a basis for concern over the consequences of chronic exposure to human health.

The rationale for structuring the document is based primarily on two major issues, exposure and response. The first portion of the document is devoted to the chlorinated benzenes in the environment: physical and chemical properties, the monitoring of the chlorinated benzenes in various media, natural and human-made sources, the transport and distribution of the chlorinated benzenes within environmental media, and the levels of exposure. The second part is devoted to biological responses in laboratory animals and humans including metabolism, pharmacokinetics, as well as the toxicological effects of the chlorinated benzenes.

This assessment is based on original publications, although the overall knowledge covered by a number of reviews and reports was also considered. The references cited were selected to reflect the current state of knowledge on those issues which are most relevant for a health assessment of the chlorinated benzenes in the environment.

2. SUMMARY AND CONCLUSIONS

2.1. SUMMARY

2.1.1. Properties, Production and Use. The chlorinated benzenes are a group of cyclic aromatic compounds in which 1-6 hydrogen atoms of a benzene ring have been replaced by up to six chlorine substitutents. This substitution yields 12 compounds: monochlorobenzene, three isomeric forms of dichlorobenzene, three isomers of trichlorobenzene, three isomers of tetrachlorobenzene, pentachlorobenzene and hexachlorobenzene. The physical properties of these compounds vary with the degree of substitution of each and are, in general, low water solubility (solubility decreasing with increasing chlorination), low flammability, moderate to high octanol/water partition coefficients (coefficients increasing with increasing chlorination) and low to moderate vapor pressures (vapor pressures decreasing with increasing chlorination). They are chemically unreactive and exist as liquids or solids at environmental conditions. Analysis of airborne chlorobenzenes are usually accomplished by adsorption onto sorbent cartridges, followed by thermal desorption and analysis by gas chromatography (GC). For water samples, the purge-trap method is used to concentrate the volatile halogenated benzenes before analysis by GC. For less volatile chlorinated benzenes, solvent extraction followed by column chromatographic cleanup of the extract and electron capture/gas chromatography (EC/GC), is the most commonly used method for the isolation, detection and quantification. Methods similar to those used for wastewater samples are commonly used for the analysis of chlorinated benzenes in biological matrices.

Annual production of these 12 chlorinated benzenes in 1983 is on the order of 450 million pounds, the majority of which is accounted for by monochlorobenzene and dichlorobenzenes. These compounds are used in a number of organic chemical syntheses, including the synthesis of other chlorobenzenes, and have applications as solvents, electrical equipment insulators, pesticides, herbicides and fungicides. Emissions of chlorobenzenes are most likely to occur during their manufacture or use as intermediates and from the disposal of waste products from manufacturing operations. Hexachlorobenzene is imported but not produced commercially in the United States, and occurs as a by-product in the synthesis of nine other chlorinated hydrocarbons; 2-5 million pounds may be generated each year.

2.1.2. Environmental Levels, Transport and Fate. Chlorinated benzenes have been identified in air, food and soil, and in surface, ground and drinking water. The highest concentrations have been found in or near manufacturing and waste disposal sites, although no study has attempted to characterize the contribution of any one source to the total environmental contamination by chlorobenzenes. Ambient air and water levels are in the microgram/cubic meter and microgram/liter range, respectively, although monitoring studies for finished water have been limited. The most frequently detected chlorinated benzenes in air and water were monochlorobenzene and the di- and trichlorobenzenes. Penta- and hexachlorobenzene have been found more frequently in food and soil, although their detection may reflect more the concern over their use as pesticides and fungicides, or their presence as contaminants in pesticides or fungicides, rather than the absence of the other chlorobenzenes.

The transport and fate of the chlorinated benzenes in the environment have not been well characterized although, from laboratory and field studies and from the known chemical and physical properties, several generalizations can be made. After emission into air, the chlorobenzenes are likely to be widely dispersed by the prevailing wind and degrade slowly through chemical and photolytic reactions. One study estimated residence times for three of the chlorobenzenes to range from 13-116 days. When released into water, these compounds, because of their low water solubility will evaporate from the surface rapidly. Small amounts are likely to remain in solution or be removed through sedimentation. Some of the chlorobenzenes can undergo microbial degradation, and all show a propensity for bioaccumulation. After release of chlorobenzenes into soil, very little will be removed by leaching with water because of low water solubility and high soil adsorption; the latter increases with the number of substituent chlorines. Evaporation is likely to occur from the upper soil layers. Overall, the less chlorinated chlorobenzenes will tend to partition from soil and water into air, there to be dispersed and degraded. The chlorobenzenes will also tend to enter the atmosphere, either as particulates or vapors, and disperse, degrade or precipitate out.

The chlorinated benzenes are lipophilic compounds that bioaccumulate in animal and human tissues from ambient air, water and food. The bioconcentration factor (BCF) (tissue concentration/media concentration) is an indicator of bioaccumulation and can be expressed in terms of such physicochemical parameters as the water solubility or the octanol/water partition coefficient which reflect the number of substituent chlorine atoms. The BCF in various fish species range from 12-46 for monochlorobenzene to >44,000 for hexachlorobenzene. Physiological exposure levels (the levels of exposure, concentration, at the site of the compounds interaction, sequestration or observed effects) are determined by absorption, metabolism, elimination and storage in adipose tissue; thus, biologically persistent compounds, such as the chlorinated benzenes, may produce prolonged physiological exposures.

No comprehensive study of human exposure to the chlorinated benzenes has been conducted, although their ubiquity in the environment and the detection of measurable residues in human tissue indicate that human exposure and absorption occur. The contribution of the chlorinated benzenes from all three media (air, water and food) to a person's total exposure cannot be made with the limited environmental monitoring data. The available data, however, indicate that human inhalation exposure to chlorinated benzenes may be higher than ingestion exposure either through drinking water or through foods.

2.1.3. **Ecological Effects.** As demonstrated in acute toxicity bioassays, the LC_{50} in fish generally decreases as the number of substituent chlorine atoms on the molecule increases (isomers vary). Chlorinated benzenes cause adverse reproductive effects in invertebrates and fish. Monochlorobenzene tested in goldfish and largemouth bass, 1,3,5-trichlorobenzene tested in brine shrimp, and the exposure of sheepshead minnows to 1,2,4,5-tetrachlorobenzene resulted in decreased hatching of eggs or embryo lethality and decreased survival of juvenile fish.

Adverse effects of chlorinated benzenes were also apparent in terrestrial organisms. Mitosis in seeds and seedlings was disrupted by 1,4-dichlorobenzene; 1,2,4,5-tetrachlorobenzene affected seed germination and seedling growth depending on soil type. Soil application rates of 224 kg/ha or higher of 1,2,4,5-tetrachlorobenzene were found to be toxic to mature cotton plants. Dichlorobenzene vapors at "saturation concentrations" inhibited the emergence of housefly pupae, while 1,2-dichlorobenzene and trichlorobenzene each in diesel oil were toxic to Douglas fir beetles.

Contact with residues of 1,3,5-trichlorobenzene shortened the lifespan of female wasps, and their eggs suffered high mortality within 7 days of exposure.

Although effects of chlorinated benzenes (mortality, decreased reproduction) on natural populations have not been adequately studied, tissue concentrations of several isomers were measured in a number of different species. Aquatic organisms (fish and invertebrates) and terrestrial species have been found to contain chlorinated benzenes levels. Tissue concentrations of the quantitated chlorinated benzenes were highest for hexachlorobenzene. The detection in North America and Europe of hexachlorobenzene in the eggs of birds and subcutaneous fat of wild animals suggests its widespread distribution in the environment.

2.1.4. **Pharmacokinetics.** Monochlorobenzene is readily absorbed through the respiratory system and the gastrointestinal tract, but the quantitative extent is not known. It is deposited in body lipids and metabolized by microsomal oxidation. Oxidative reactions lead to the formation of arene oxides; these epoxides are metabolized further to the ortho-, meta- or para-chlorophenols. The chlorophenols may conjugate with glutathione and be detoxified by conversion to the corresponding mercapturic acids and excreted in the urine or they may bind to cellular proteins. Binding to cellular protein appears to be correlated with necrotic pathological changes in the kidneys and livers of rodents. In addition to conjugation with glutathione, metabolites of monochlorobenzene (monophenols and diphenols) can conjugate with glucuronic acid or with sulfate and be excreted in the urine. Monophenols are the major metabolites; the diphenols are formed to a lesser degree. The arene oxides, 3-chlorobenzene oxide or 4-chlorobenzene oxide, also can be converted to the dihydrodiol by epoxide hydrase and dehydrogenated to form chlorocatechols. There appear to be species differences in the profile of urinary conjugation of metabolites, and end metabolites may vary depending on the availability of tissue glutathione. Detoxification by conjugation with glutathione is important in the modulation of toxic effects especially at high exposure levels.

The available data for rats, rabbits and humans indicate that the dichlorobenzenes are absorbed through the lungs, gastrointestinal tract and intact skin, though actual determinations of absorption rates were not located. Once absorbed through either inhalation or ingestion, the dichlorobenzenes are rapidly distributed to many tissues, including blood, adipose, kidney, liver, lung, heart, brain and muscle tissues. Distribution is primarily to adipose tissue, which has initial levels 10-32 times the blood concentrations and to lung and kidney tissues to a greater extent than liver, muscle and plasma. Single-dose and repeated exposures by both inhalation and ingestion show similar patterns of distribution. Elimination of the dichlorobenzenes and their metabolites occurs within 5-6 days after exposure, although elimination from adipose tissue is slowest and 1,2-dichlorobenzene and metabolites are eliminated slightly more rapidly than 1,4-dichlorobenzene. The dichlorobenzenes are primarily metabolized by hydroxylation to their respective dichlorophenols, which are excreted in the urine in the form of glucuronic and sulfate conjugates. Some metabolites are excreted in the bile, although the majority are then reabsorbed by the enterohepatic pathway and reexcreted in the urine. Intermediates of the metabolism of 1,4-dichlorobenzene, possibly arene oxides, bind to liver protein and may be involved in the induction of hepatotoxicity.

The limited comparative pharmacokinetic data available on the trichlorobenzenes prevent specification of the absorption, distribution, metabolism and excretion of the individual isomers. From the available data, it appears that metabolism in at least three species has a common first step, the production of an arene oxide intermediate. Subsequent metabolic steps, however, vary among the species examined, at least for the most studied isomer, 1,2,4-trichlorobenzene.

In general, the pharmacokinetics of the trichlorobenzenes are similar to those described for the other halogenated aromatics. These compounds are lipophilic and their metabolism and excretion depend on conversion to polar intermediates. In addition, their lipophilic character provides for ready absorption from the gastrointestinal tract and initial distribution to the more highly perfused tissues, particularly the liver, after which they are either metabolized and excreted or redistributed to adipose tissue or skin. Additional experiments are needed to clarify the relationship of these studies to the metabolism of trichlorobenzenes in humans.

No studies describing the absorption, distribution, metabolism or excretion of 1,2,3,4-, 1,2,3,5- or 1,2,4,5-tetrachlorobenzene following inhalation exposure were located in the available literature. The pharmacokinetics of the tetrachlorobenzene isomers following oral administration is well characterized in rabbits, but not in other animal species. The lipophilic characteristics of the tetrachlorobenzene isomers would allow efficient transepithelial absorption at the gastrointestinal and respiratory surfaces. Once absorbed, the tetrachlorobenzene isomers administered orally to rabbits were rapidly accumulated in fat, metabolized primarily to tetrachlorophenols and conjugated partly as glucuronides and ethereal sulfates or eliminated unchanged in the expired air or feces.

No pharmacokinetic data were available for humans, except a report of 1,2,4,5-tetrachlorobenzene in adipose tissue (range of 0.006-0.039 mg/kg bw; mean of 0.019 mg/kg bw) of 15 Tokyo residents. The tetrachlorobenzene isomers are both *in vivo* and *in vitro* metabolites of the pesticides, lindane and hexachlorobenzene; therefore, human exposure via air, food and drinking water may occur from the environmental degradation of these pesticides.

Although studies of the absorption of pentachlorobenzene indicated that absorption does occur through the gastrointestinal tract, the rate or extent of absorption has not been determined. A study in rabbits indicated that up to 50% of a dose was absorbed within 3-4 days. Oral administration to monkeys indicated 95% absorption within 4 days. Absorption resulting from inhalation has not been studied, and absorption from dermal exposure was found to be rather poor in rats. Once absorbed, pentachlorobenzene is distributed to many tissues, with the highest levels appearing in fat, liver and bone marrow. A study in rats demonstrated that transport across placental membranes occurred readily and that accumulation of pentachlorobenzene in the fetus is highest in the liver. No studies were encountered that described the distribution of pentachlorobenzene after inhalation or dermal exposure.

The metabolism of pentachlorobenzene is not fully understood, but some studies suggested that metabolic activity other than the hepatic cytochrome P-450, xenobiotic metabolizing system may be involved. Metabolism appeared to be primarily via oxidation to two major metabolites, pentachlorophenol and 2,3,4,5-tetrachlorophenol, which were excreted in the urine. Metabolism and excretion occurred at a slow rate; an estimated elimination half-life for a single dose in primates was 2-3 months.

The pharmacokinetics of hexachlorobenzene in a number of mammalian species have been studied in detail following oral administration and, to a lesser extent, following intravenous or intraperitoneal injection. No information was present in the available literature on hexachlorobenzene metabolism following inhalation or topical application. Absorption of hexachlorobenzene from the intestinal tract appears to depend on the vehicle used during test material administration. Thus, when hexachlorobenzene is administered in olive oil, ~80% of the dose is absorbed; when it is administered in an aqueous solution, in 1% methyl cellulose or in a crystalline form, relatively little (<20%) is absorbed. Intestinal absorption of hexachlorobenzene occurs primarily through lymphatic channels, with only a minor portion being absorbed into the portal circulation.

Following absorption, hexachlorobenzene is distributed to tissues that have a high lipid content. The adipose tissue accumulates the greatest concentrations of hexachlorobenzene in all species studied, although bone marrow and skin, which contain large amounts of lipids, also accumulate hexachlorobenzene. The adrenal cortex accumulates hexachlorobenzene at concentrations approaching those of fat. Other body constituents (e.g., kidneys, lungs, heart, spleen and blood) generally contain much lower amounts of hexachlorobenzene. Intravenous injection of hexachlorobenzene results in a tissue distribution similar to that seen following oral administration. Hexachlorobenzene is transported via the placenta and is distributed in fetal tissue as indicated by studies in rabbits, rats, mice, mink and ferrets.

Hexachlorobenzene is metabolized slowly into other chlorinated benzenes, chlorinated phenols and other minor metabolites and forms glucuronide and glutathione conjugates. Tissues were found to contain mainly unchanged hexachlorobenzene together with small amounts of metabolites. Similarly, only small amounts of hexachlorobenzene metabolites were detected in feces, whereas most of the metabolites were excreted in the urine together with small amounts of unchanged hexachlorobenzene. There are indications that females produce and excrete more hexachlorobenzene metabolites than do males.

The excretion of hexachlorobenzene from treated animals is slow and occurs mainly through the feces with relatively little being excreted in the urine. It is characterized by an initial rapid phase followed by a very slow phase. This slow phase of excretion can be enhanced by the administration of mineral oil, paraffin or n-hexadecane. Both biliary and intestinal excretion contribute to fecal excretion. A three-compartment mammillary model has been reported for the behavior of hexachlorobenzene in beagles and rhesus monkeys following i.v. injection of a single dose. Radioactivity was not detected in exhaled air following i.p. injection of ^{14}C-hexachlorobenzene. Hexachlorobenzene has been detected in the milk of nursing mammals.

2.1.5. Effects on Humans. No epidemiologic studies regarding the effects of exposure to monochlorobenzene are available. Human exposure to monochlorobenzene by inhalation or by accidental ingestion can cause neurotoxic effects. It is not known if the effects are reversible after long-term exposure or if there are other sites of toxicity.

Epidemiologic data on dichlorobenzenes are insufficient to evaluate dose-response association. Possible chronic effects of exposure to the dichlorobenzenes are indicated by case reports of the chronic exposure of individuals, i.e., repeated exposures over a period of more than a year, suggesting a common set of toxic effects, those of the reticuloendothelial and hematopoietic systems and those of the liver. Of the 23 exposure cases found in the literature, 17 involved pathological changes in the blood or liver, including chronic lymphoid leukemia, acute hemolytic anemia, aplastic anemia and bone marrow hyperplasia. Although the exposures in these cases are not well defined in time and often involve other toxic substances, together they suggest a common pathologic action of the dichlorobenzenes on bone marrow and other organs of the blood-forming system. The one available epidemiologic study supports this generalization in that the reported short-term exposure to 1,2-dichlorobenzene (8 hours/day for 4 days) produced alterations in the chromosomes of leukocytes. This study did not establish an association between chromosomal alterations and the pathologic changes that characterize the case studies.

Human exposure to 1,2,4-trichlorobenzene at 3-5 ppm causes eye and respiratory irritation. The only other data on human exposure are individual case reports of aplastic anemia of persons exposed occupationally or domestically.

Only one epidemiologic study was available regarding the effects of the tetrachlorobenzenes on humans and this study examined peripheral lymphocytes for chromosomal abnormalities in blood. The blood was collected from Hungarian workers engaged in the production of 1,2,4,5-tetrachlorobenzene. There were observed chromosome aberrations in the lymphocytes; however, no airborne concentrations or exposures were determined.

No epidemiologic or case studies of effects on humans resulting from exposure to pentachlorobenzene were available for review.

A few epidemiologic studies with occupationally-exposed workers have been reported, together with studies conducted in Turkey and in the United States (i.e., Louisiana) on the general population following accidental exposure to hexachlorobenzene. These studies qualitatively support the toxicity of hexachlorobenzene, but give little dose-response information.

Biological monitoring of plasma levels clearly show more hexachlorobenzene in the plasma of exposed compared to nonexposed individuals, although no biologically significant adverse health effects were seen during the observation periods. The exposure of humans to hexachlorobenzene in Turkey during 1955-1959 caused an epidemic of hexachlorobenzene-induced porphyria cutanea tarda (PCT), also known as porphyria turcica, which is manifested by disturbed porphyrin metabolism, cutaneous lesions and hyperpigmentation. The authors estimated that from 0.05-0.2 g/day were ingested. In exposed children under 1 year of age, pink sore was observed as well as 95% mortality in these infants.

Follow-up studies conducted with patients 20-25 years after the onset of porphyria showed that a few subjects still had active porphyria, whereas >50% exhibited hyperpigmentation scarring, as well as other dermatologic, neurologic and skeletal features of hexachlorobenzene toxicity. Hexachlorobenzene residues were also found in the blood, fat and breast milk of some patients.

A correlation was found between hexachlorobenzene levels in blood and the number of years worked in a chlorinated solvents plant. The concentration of urinary uroporphyrins and coproporphyrins ranged from 21-37 and 67-101 $\mu g/\ell$, respectively, for the period between 1974 and 1977. An epidemiologic survey conducted with 86 residents in the vicinity of this chlorinated solvents plant showed elevated hexachlorobenzene residues in plasma. Higher levels of hexachlorobenzene residues were found in males than in females, but these were not associated with race or food consumption.

2.1.6. **Mammalian Toxicology.** Acute exposure to monochlorobenzene by inhalation causes sensory irritation of the respiratory system after a few minutes; exposure for several minutes to several hours causes narcosis and central nevous system depression, which can result in death. Monochlorobenzene is also toxic by the oral or parenteral routes. Systemic effects of acute toxic doses include kidney damage. Subchronic inhalation exposure at 1.0 mg/m^3 (continously for 60 days) causes neurotoxic effects in rats, an increase in blood cholinesterase and abnormal chronaxia of the muscles. Repeated exposure of rats to monochlorobenzene at 250 ppm (1157 mg/m^3) causes slight changes in the liver, kidneys and adrenal cortex. Repeated oral dosing of rats or dogs (100-200 mg/kg/day) causes some toxic manifestation in the liver and kidneys. Gavage administration of monochlorobenzene to mice and rats 5 times/week for 13 weeks resulted in increased mortality in the higher dose groups ($\geq$250 mg/kg), urinary porphyria and dose-dependent injury to the liver, kidney, bone marrow, spleen and thymus. A set of similar studies were conducted in mice and rats for 2 years and resulted in some increased mortality in the male monochlorobenzene exposed groups when compared with controls. Only equivocal evidence for mild monochlorobenzene-induced hepatocellular necrosis was found in rats.

Although one study in _Streptomyces antibioticus_ found monochlorobenzene to induce reversion to vitamin B_1 prototrophy and one study in _Saccharomyces cerevisiae_ showed induction of DNA damage, several other studies using bacterial, fungal and mammalian tissue culture systems were negative. The carcinogenic activity of monochlorobenzene was tested by the NTP bioassay program in two rodent species at doses of 60 and 120 mg/kg bw/day in male and female rats and female mice, and at 30 and 60 mg/kg bw/day in male mice. Carcinogenicity was not demonstrated for monochlorobenzene in this study, but high dose male rats had a significant increase in neoplastic nodules of the liver.

Repeated exposures to monochlorobenzene at 2.0 mg/ℓ (vapors) or 0.250 mg/kg/day (oral) were found to cause atrophy of the epithelial tissue in the seminiferous tubules and decreased spermatogenesis in dogs and rats and increased gonad weight/body weight ratios in female rats.

Studies of the acute and subchronic toxicity of the dichlorobenzene isomers indicate that generally these compounds have similar target organs and effects. At oral doses ranging from 125-1000 mg/kg over periods of up to 6 months, the dichlorobenzenes cause central nervous system depression, injury to liver, kidney, heart, thymus and spleen, and hepatic porphyria; however, one study reported that a dose of 0.01 mg/kg over a 5-month period inhibited erythropoiesis and bone marrow activity. The subchronic oral toxicity studies in rats provide two estimates of no-observed-effect level (NOEL) values: 0.001 mg/kg for 1,4-dichlorobenzene and 18.8 mg/kg for 1,2- and for 1,4-dichlorobenzene. The National Toxicology Program (NTP, 1982) subchronic oral study on 1,2-dichlorobenzene in mice provided higher estimated NOEL values of 125 and 250 mg/kg for males and females, respectively. A 2-year NTP chronic oral gavage study on 1,2-dichlorobenzene in rats and mice, conducted primarily as a carcinogenesis bioassay at the 60 and 120 mg/kg dose levels, resulted only in increased mortality in the male rats given 120 mg/kg. Acute and subchronic inhalation studies of dichlorobenzenes indicate similar toxic effects and target sites as seen in the oral studies. The effects occurred at doses $\geq$950 mg/m^3; inhalation NOELs were reported as 580 mg/m^3 for 1,2-dichlorobenzene and 290 mg/m^3 for 1,4-dichlorobenzene.

Studies of the mutagenic activity of dichlorobenzenes show little or no activity in a range of bacterial systems, including Salmonella, with and without metabolic activation. However, these studies were lacking in experimental detail. Several studies with mold and plant cultures treated with dichlorobenzenes have reported mutations and chromosomal alterations. The carcinogenic activity of one isomer, 1,2-dichlorobenzene, was tested in the

NTP bioassay program in two rodent species at doses of 60 and 120 mg/kg. No evidence of carcinogenic activity was found under the test conditions.

The effects in mammals of acute exposure by various routes to trichlorobenzenes include local irritation, convulsions and death. Livers, kidneys, adrenals, mucous membranes and brain ganglion cells appear to be target sites with effects including edema, necrosis, fatty infiltration of livers, increased organ weights, porphyrin induction and microsomal enzyme induction.

Quantitative data on the toxic effects of trichlorobenzene following subchronic exposure by various routes were obtained in a variety of species. In general, these studies indicate that the liver and kidney are target organs. Inhalation of 1,2,4-trichlorobenzene at ≥ 74.2 mg/m^3 (10 ppm) for 6 hours/day, 5 days/week for up to 26 weeks induced hepatocytomegaly and hyaline degeneration in several species, although these effects may be to some extent reversible. One study identified 22.3 mg/m^3 (3 ppm) as a no-observed-adverse-effect level (NOAEL) in rats, while another study reported that some rats exposed by inhalation to 1,3,5-trichlorobenzene at 7423 mg/m^3 (1000 ppm) for 13 weeks showed squamous metaplasia and focal hyperplasia of the respiratory epithelium, which appeared to be reversible. Subchronic oral studies have also found that the trichlorobenzenes induce hepatic xenobiotic metabolism and porphyria. Subchronic dermal exposure resulted in mild to moderate irritation.

One chronic study on the effects of trichlorobenzene (0.03 mℓ) painted on the skin of mice for 2 years reported increased mortality in females at the low dose (30% solution in acetone) and in both sexes at the high dose (60% solution).

Results of two reports on mutagenicity tests with Salmonella typhimurium test strains were negative. However, this test system is generally insensitive to chlorinated compounds. One carcinogenicity study, a 2-year skin painting study in mice, failed to demonstrate a tumorigenic effect. A multigeneration study of the reproductive effects of oral exposure of rats to trichlorobenzene and an oral teratogenicity study in rats failed to show effects on reproduction or fetal development, although pups had mild osteogenic changes.

The only mammalian toxicology data available for tetrachlorobenzenes are the result of oral exposures. The oral LD_{50} for 1,2,4,5-tetrachlorobenzene was reported as 1035 mg/kg in mice and 1500 mg/kg in rats and rabbits when administered in sunflower oil and 2650 mg/kg in mice when administered in 1.5% starch solution. Subchronic oral exposure of rats and rabbits to 1,2,4,5-tetrachlorobenzene resulted in statistically significant effects on biochemical parameters, including reticulocytosis, increased blood cholinesterase activity, erythremia and an indication that glycogen formation was impeded; at higher doses of 1,2,4,5-tetrachlorobenzene, rats also had increased kidney and liver weights, and renal and hepatic histologic changes.

Reversible effects on serum alkaline phosphatase and total bilirubin were reported in dogs given 5 mg/kg bw/day 1,2,4,5-tetrachlorobenzene in the diet for 2 years.

1,2,4,5-Tetrachlorobenzene was not mutagenic in the sex-linked recessive lethal assay with Drosophila melanogaster. However, because only an abstract of the study was available, experimental details were too sparse to permit an evaluation of this negative result. Both 1,2,3,5- and 1,2,4,5-tetrachlorobenzenes were negative in the reverse mutation assay with Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538. These results were reported in an abstract with insufficient experimental detail. Also, a negative result for chlorinated compounds in the Salmonella reversion assay is not unexpected.

No information was available regarding the carcinogenicity of any of the three tetrachlorobenzene isomers in either animals or humans.

The tetrachlorobenzene isomers have been found to induce appreciable maternal toxicity, mild fetotoxicity and negligible teratogenicity in rats following oral administration.

Oral LD_{50} values were determined for pentachlorobenzene in adult rats (1080-1125 mg/kg) and mice (1175-1370 mg/kg), and for weanling rats (940 mg/kg). No clinical signs of toxicity were observed in adult rats following dermal application of 2500 mg/kg pentachlorobenzene. Also, it was demonstrated that pentachlorobenzene caused an increase in the liver content of cytochrome P-450, microsomal drug metabolizing enzymes and microsomal proteins.

A subchronic feeding study indicated that the primary toxic effects are on the liver and kidneys, although slight changes in some hematologic parameters (e.g., decreased erythrocyte count, hemoglobin and hematocrit; and increased leukocyte count) occurred in the high dose groups. Histologic examination identified pathologic changes in the livers of the female rats fed 500 and 1000 ppm for 180 days and in the 1000 ppm male rats treated for 100 days. These data were sufficient to identify a subchronic lowest-observed-adverse-effect level (LOAEL) of 500 ppm (~27-63 mg/kg/day) and a NOEL of 250 ppm (~16-31 mg/kg/day).

No mutagenic activity was detected in five strains of Salmonella typhimurium when tested at five unspecified concentrations of pentachlorobenzene in the presence and absence of rat liver microsomes induced by Aroclor 1254. These results were reported in an abstract with insufficient experimental details presented. A negative result is not unexpected, because the Salmonella test system is generally insensitive to chlorinated compounds.

Studies also have shown that pentachlorobenzene is capable of causing reproductive and developmental effects. Female rats fed diets containing pentachlorobenzene during mating and gestation produced litters with reduced pup survival and body weights at weaning, and increased liver-to-body weight ratios. No adverse effects were observed in the offspring of the dams exposed to 125 ppm (6-16 mg/kg/day).

Single oral doses of pentachlorobenzene given daily to pregnant rats during gestation increased the incidence of fetal death at all tested doses, identifying a LOAEL of 50 mg/kg/day. Sternal defects and an increase in the incidence of extra ribs also were observed at doses of 200 mg/kg/day and 50, 100 and 200 mg/kg/day, respectively.

In a study of possible reproductive and teratogenic effects, doses of 50 and 100 mg/kg/day of pentachlorobenzene administered by gavage to pregnant mice had no adverse effect on fetal development or survival of the pups.

The acute oral toxicity of hexachlorobenzene has been found to be low with LD_{50} values ranging from 1700-10,000 mg/kg. Subchronic oral toxicity studies with a number of mammalian species indicated a significant increase in liver and kidney weights in hexachlorobenzene-treated animals. Studies have shown increases in other organ weights as well. The livers from hexachlorobenzene-exposed animals have shown histologic changes such as irregular shaped and moderately enlarged liver mitochondria and increases in the size of the centrilobular hepatocytes. Chronic toxicity studies revealed the same type of effects seen in the subchronic studies, plus hexachlorobenzene associated life-shortening and various hepatic and renal pathologies. These subchronic and chronic effects were usually dose-related. Other effects included multiple alopecia and scabbing, together with neurologic effects in rats, mice and dogs. A dose-related histopathologic change in the ovaries of monkeys has also been reported.

Increased porphyrin levels in the liver and in urine have been reported for all species studied except the dog. Hexachlorobenzene was found to cause the accumulation of β-H-steroids which induce porphyrin biosynthesis and to inhibit uroporphyrinogen decarboxylases. The inhibition of uroporphyrinogen decarboxylase appears to be from pentachlorophenol, a hexachlorobenzene metabolite. Indications are that females are more susceptible to hexachlorobenzene-induced porphyria than are males, which may be related to the females estrogen levels and greater hexachlorobenzene metabolism. Hexachlorobenzene was reported to produce a mixed-type induction of cytochromes resembling that produced by a combination of phenobarbital (P-450) and 3,4-benzpyrene (P-448). In addition, the activities of several hepatic microsomal enzymes were found to be induced by hexachlorobenzene.

Hexachlorobenzene did not induce dominant lethal mutations in two studies but was reported to be mutagenic in a yeast, S. cerevisiae, assay at a concentration of 100 ppm. Hexachlorobenzene possessed no detectable levels of mutagenic activity in the Salmonella histidine reversion assay. The chronic toxicity studies provide sufficient evidence of the carcinogenicity of hexachlorobenzene in animals since there was an increased incidence of malignant tumors of the liver in two species (haemangioendothelioma in hamsters and hepatocellular carcinoma in rats) as well as reports of hepatoma in mice, rats and hamsters. Hexachlorobenzene given to pregnant mice was found to produce cleft palates and renal agenisis in exposed pups. Certain chemicals were found to alter the toxicity of hexachlorobenzene in mammals, whereas hexachlorobenzene pretreatment was reported to increase CCl_4 toxicity and alter the immune responses of treated animals.

2.2. CONCLUSIONS

The chlorinated benzenes are a group of 12 cyclic aromatic compounds in which 1-6 hydrogen atoms of a benzene ring have been replaced by up to six chlorine substituents. As the benzene ring is increasingly chlorinated there are physiochemical trends towards increased melting points, boiling points, densities and log partition coefficients, and decreased volatility and water solubility of the compounds.

A wide range and severity of chlorinated benzenes-induced health effects have been reported in rodents and other laboratory animals. Some of these same effects have also been observed in chlorinated benzenes-exposed humans as well, but the human reports are not as extensive or complete as the animal studies. A review of the animal chlorinated benzenes health effects literature also indicates that there are some large data gaps existing for several of the chlorinated benzene isomers, especially for 1,3-dichlorobenzene, the trichlorobenzenes and the tetrachlorobenzenes. The animal studies indicate a trend of increasing toxicity with increased chlorination of the benzene ring, e.g., hexachlorobenzene is more porphyrinogenic than monochlorobenzene. Adequate evidence of the carcinogenicity of the different chlorinated benzenes has only been shown for hexachlorobenzene. Hexachlorobenzene has been classified as a probable carcinogen in humans.

2.3. NEEDS FOR FUTURE RESEARCH

- Further studies should be conducted to determine detailed pharmacokinetics of each of the chlorinated benzene isomers (i.e., absorption, distribution, metabolism and excretion).
- Further studies should be conducted to determine more thoroughly the long-term toxicity and, in some cases, the carcinogenicity of many of the chlorinated benzene isomers, except for hexachlorobenzene where sufficient data already exists.
- Further mutagenicity studies should be conducted on those chlorinated benzene isomers which do not have sufficient mutagenicity data available.

- Studies should be conducted to assess the potential of the chlorinated benzenes to cause DNA damage.

- Teratogenicity, fetotoxicity and reproductive studies should be conducted using various routes of exposure, with emphasis on the inhalation route, on all the chlorinated benzene isomers.

- Studies on the neurotoxic effects of the chlorinated benzene isomers should be conducted using various routes of exposure, with emphasis on the inhalation route.

- Studies should be conducted to assess for possible chlorinated benzenes effects on alterations to the endocrine, hematopoietic and immunologic systems in humans and animals.

- Further studies need to be conducted on the porphyria-producing properties of the chlorinated benzenes [i.e., the properties of the chlorinated benzene molecules or their metabolite(s) which are responsible for this adverse health effect in humans and animals].

- Investigations need to be conducted into the quantitative structure-activity relationships of the chlorinated benzenes with an effort to relate biological and health effects to physiochemical properties.

- Studies are needed to identify the extent of human exposure from each of the chlorinated benzene isomers and the relative contribution of the various environmental medias to the total human exposure.

- Exposure and health assessments of indoor air pollution by chlorinated benzenes need to be made. This is important especially for the dichlorobenzenes which are present in household space deodorants and moth repellants.

- Epidemiologic studies need to be conducted on individuals who are occupationally exposed to the chlorinated benzenes, with particular emphasis on those adverse health effects already observed in the human and animal studies.

- Further follow-up studies are needed concerning the health of the Turkish individuals who were exposed to hexachlorobenzene in the 1950's, with particular emphasis on their cancer incidences.

3. PHYSICAL AND CHEMICAL PROPERTIES/ANALYTICAL METHODOLOGY

The chlorinated benzenes are the group of substituted benzene compounds in which 1-6 hydrogen atoms of benzene are replaced by chlorine atoms with no substituents present other than chlorine and hydrogen. The chlorination of benzene can yield 12 different compounds: monochlorobenzene (C_6H_5Cl); 1,2-, 1,3- and 1,4-dichlorobenzene ($C_6H_4Cl_2$); 1,2,3-, 1,2,4- and 1,3,5-trichlorobenzene ($C_6H_3Cl_3$); 1,2,3,4-, 1,2,3,5- and 1,2,4,5-tetrachlorobenzene ($C_6H_2Cl_4$); pentachlorobenzene (C_6HCl_5); and hexachlorobenzene (C_6Cl_6). The chemical structures of these compounds are shown in Figure 3-1.

3.1. SYNONYMS, TRADE NAMES AND IDENTIFICATION NUMBERS

Synonyms, trade names and identification numbers for the 12 chlorinated benzenes are listed in Table 3-1.

3.2. PHYSICAL AND CHEMICAL PROPERTIES

Some physical properties of the chlorobenzenes are shown in Tables 3-2 and 3-3. In general, the chlorinated benzenes have low water solubility, moderate to high octanol/water partition coefficients and low to moderate vapor pressures at 25°C, and low flammability. Apart from hexachlorobenzene, they are considered to be volatile compounds because their Henry's Law constants are greater than 10^{-4} atm m^3 q $\cdot$ mol^{-1} (MacKay et al., 1979).

The chlorobenzenes are chemically very unreactive compounds and are generally stable under ambient conditions in the laboratory. Because of the electron-withdrawing character of the chlorine atom relative to carbon, the chlorobenzenes are highly resistant to electrophilic attack (e.g., chlorination), and each additional chlorine substituent further lowers the reactivity of these compounds. Hydroxylations occur only at high temperatures in

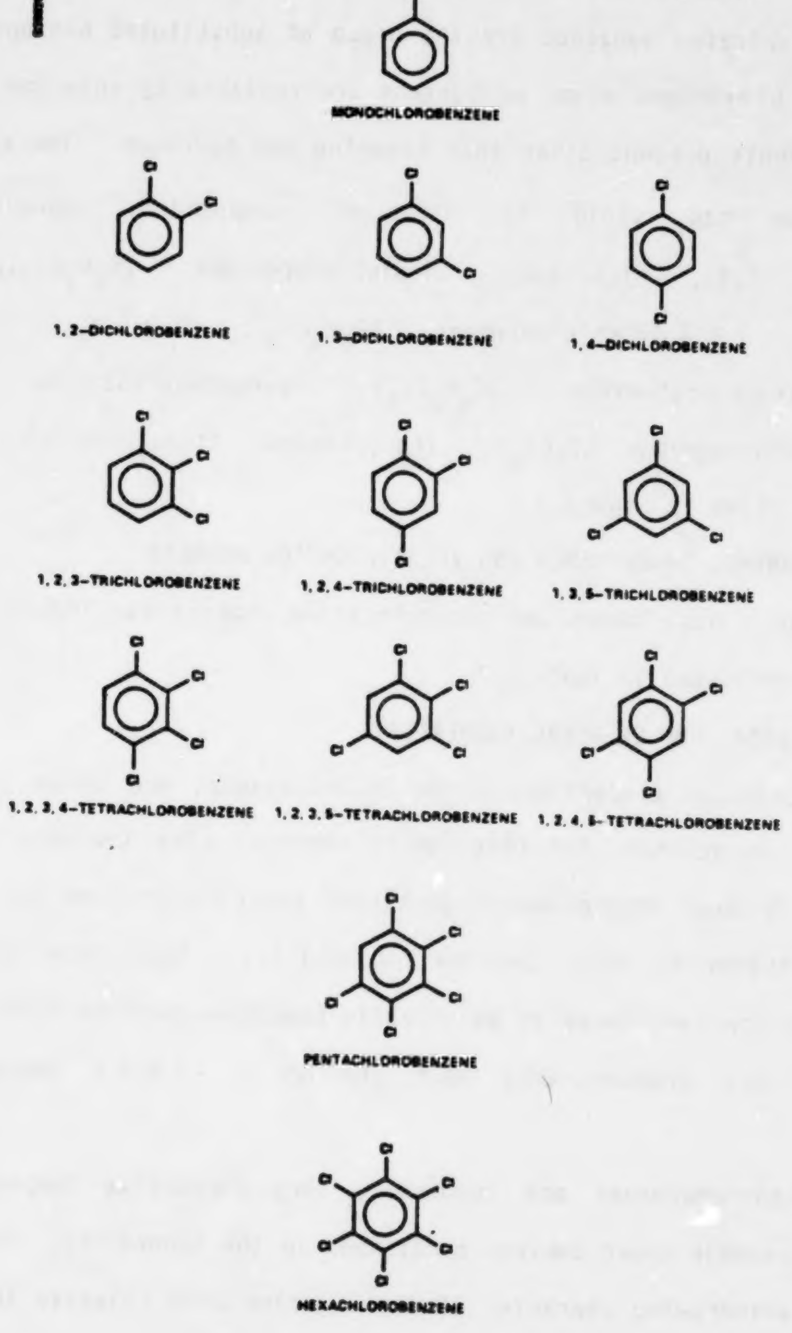
Cl
MONOCHLOROBENZENE
1, 2-DICHLOROBENZENE
1, 3-DICHLOROBENZENE
1, 4-DICHLOROBENZENE
1, 2, 3-TRICHLOROBENZENE
1, 2, 4-TRICHLOROBENZENE
1, 3, 5-TRICHLOROBENZENE
1, 2, 3, 4-TETRACHLOROBENZENE
1, 2, 3, 5-TETRACHLOROBENZENE
1, 2, 4, 5-TETRACHLOROBENZENE
PENTACHLOROBENZENE
HEXACHLOROBENZENE

FIGURE 3-1

Chemical Structure of the Chlorinated Benzenes

TABLE 3-1

Synonyms, Trade Names and Identification Numbers of the Chlorinated Benzenes*

Chemical	Identification Number	Synonyms and Trade Names
Monochlorobenzene	CAS No. 108-90-7	Chlorobenzene
	TSL No. CZ017500	Benzene chloride
	NCI No. C54886	Phenyl chloride
	EPA Haz Waste No. U037	Chlorobenzol
	EPA Haz Waste No. F002	MCB
	DOT Haz Mat No. UN1134	Chlorbenzene
		Monochlorbenzene
		Benzene, chloro-
		Chlorobenzeen (Dutch)
		Chlorobenzol
		Chlorobenzene (Polish)
		Clorobenzene (Italian)
		Monochlorobenzene (Dutch)
		Monochlorobenzol (German)
		Monochlorobenzene (Italian)
Dichlorobenzene		
1,2-	CAS No. 95-50-1	o-Dichlorobenzene
	TSL No. CZ4500000	o-Dichlor benzol
	NCI No. C54944	DCB
	EPA Haz Waste No. U070	Dowtherm E
	EPA Haz Waste No. F002	ODB
	DOT Haz Mat No. UN1591	o-DCB
		o-Dichlorobenzol
		Orthodichlorobenzene
		Orthodichlorobenzol
		Chloroben
		Dizene
		Dichlorobenzene, ortho, liquid
		Special Termite Fluid
		Termitkil
		Cloroben
		Benzene, 1,2-dichloro-
		Benzene, o-dichloro-
		ODCB
		Dilantin DB

TABLE 3-1 (cont.)

Chemical	Identification Number	Synonyms and Trade Names
Dichlorobenzene		
1,3-	CAS No. 541-73-1 EPA Haz Waste No. U071	Benzene, *m*-dichloro- Benzene, 1,3-dichloro- *m*-Phenylene dichloride *m*-Dichlorobenzol *m*-Dichlorobenzene *meta*-Dichlorobenzene
1,4-	CAS No. 106-46-7 TSL No. C24550000 NCI No. C54955 EPA Haz Waste No. U072 DOT Haz Mat No. UN1592	Di-chloricide Paramoth *p*-Dichlorobenzene PDB Paracide Paradichlorobenzene Paradi Paradow Santochlor *p*-DCB *p*-Dichlorobenzeen (Dutch) 1,4-Dichloorbenzeen (Dutch) *p*-Dichlorbenzol (German) 1,4-Dichlor-benzol (German) *p*-Dichlorobenzol Dichlorobenzene, para, solid 1,4-Dichlorobenzene (Italian) *p*-Diclorobenzene (Italian) para Crystals Paradichlorobenzol Paranuggets Parazene Benzene, *p*-dichloro- Benzene, 1,4-dichloro- *p*-Chlorophenyl chloride Evola Persia-Perazol

TABLE 3-1 (cont.)

Chemical	Identification Number	Synonyms and Trade Names
Trichlorobenzene		
1,2,3-	CAS No. 87-61-6	*vic*-Trichlorobenzene 1,2,6-Trichlorobenzene *v*-Trichlorobenzene
Trichlorobenzene		
1,2,4-	CAS No. 120-82-1 TSL No. DC2100000	Benzene, 1,2,4-trichloro- asym-Trichlorobenzene TCB Trojchlorobenzen (Polish) 1,2,4-Trichlorobenzol Hostetex L-Pec
1,3,5-	CAS No. 108-70-3	s-Trichlorobenzene sym-Trichlorobenzene TCB TCBA Benzene, 1,3,5-trichloro-
Tetrachlorobenzene		
1,2,3,4-	CAS No. 634-66-2	Benzene, 1,2,3,4-tetrachloro-
1,2,3,5-	CAS No. 634-90-2	Benzene, 1,2,3,5-tetrachloro-
1,2,4,5-	CAS No. 95-94-3 TSL No. DB9450000 EPA Haz Waste No. U207	Benzene tetrachloride Benzene, 1,2,4,5-tetrachloro- s-Tetrachlorobenzene
Pentachlorobenzene	CAS No. 608-93-5 EPA Haz Waste No. U183 TSL No. DA6640000	1,2,3,4,5-Pentachlorobenzene QCB Benzene, pentachloro- Quintochlorobenzene

TABLE 3-1 (cont.)

Chemical	Identification Number	Synonyms and Trade Names
Hexachlorobenzene	CAS No. 118-74-1 TSL No. DA2975000 EPA Haz Waste No. U127	Esaclorobenzene (Italian) Amatin Anticarie Bunt-Cure Bunt-No-More Co-op Hexa Granox NM HCB HEXA C.B. Hexachlorobenzol (German) Hexachlorobenzene Julin's Carbon Chloride No Bunt No Bunt 40 No Bunt 80 No Bunt Liquid Pentachlorophenyl Chloride Perchlorobenzene Phenyl Perchloryl Sanocide Smut-Go Snieciotox

*Source: National Library of Medicine (NLM), Toxicology Data Bank (TDB)

TABLE 3-2

Physical Properties of the Chlorinated Benzenes[a]

Chemical	Molecular Weight	Melting Point (°C)	Boiling Point[b] (°C)	Density[c] (g/mℓ)	Henry's Law Constant[d] x 10^3 (atm m^3 mol^{-1})	Log p^o[d]	Water Solubility (mg/ℓ)[e]	Flash Point (°C or °F)	Index of Refraction at (°C)
Monochlorobenzene	112.56	-45.6	132	1.1	2.6	2.84[f]	500(20)[g]	85 F/cc[h]	1.5241(20)
Dichlorobenzene									
1,2-	147.01	-17.0	180.5	1.30	1.3	3.38[f]	145[g]	151 F/cc	1.5515(20)
1,3-	147.01	-24.7	173	1.28(25)		3.38[f]	123[g]	NA	1.5459(20)
1,4-	147.01	53.1	174	1.25	2.4	3.39[f]	79[g]	150 F/cc	1.5285(60)
Trichlorobenzene									
1,2,3-	181.46	52.6	221	1.69	1.0	4.1[i,j]	31.5[k]	113 C	1.5776(19)
1,2,4-	181.46	16.95	213.5	1.45	4.3	4.12[l]	34.6[k]	110 C	1.5717(20)
1,3,5-	181.46	63.4	208.4	1.39(64)[m]		NA	6.6[k]	107 C	1.5662(19)
Tetrachlorobenzene									
1,2,3,4-	215.90	47.5	254	NA		NA	4.3[k]	NA	NA
1,2,3,5-	215.90	54.5	246	NA		NA	3.5[k]	311 F	NA
1,2,4,5-	215.90	139.5	246	1.86(22)		4.93[j,n]	0.60[k]	311 F	NA
Pentachlorobenzene	250.34	86	277	1.83(16.5)		5.63[n]	0.56[k]	NA	NA
Hexachlorobenzene	284.76	230	3229	1.57(23)	0.12	5.8[l]	0.005[k]	468 F	NA

[a] Data are from the National Library of Medicine (NLM), Toxicology Data Bank (TDB), except as noted.

[b] At 760 mm

[c] At 20°C, except as noted

[d] MacKay et al., 1979

[e] At 25°C, except as noted

[f] Leo et al., 1971

[g] Verschueren, 1977

[h] These are data from closed cup (cc) experiments

[i] Monsanto, 1978

[j] Isomer unspecified

[k] Yalkowsky and Valvani, 1980

[l] Hansch and Leo, 1981

[m] Horvath, 1982

[n] U.S. EPA, 1980b

P^o = Partition coefficient at 25°C

NA = Not available

TABLE 3-3

Vapor Pressures and Vapor Densities of the Chlorinated Benzenes

Chemical	Vapor Pressure (mm Hg)	Specific Vapor Density (air = 1)
Monochlorobenzene	8.8 at 20°C[a] 10 at 22.2°C[b] 11.8 at 25°C[b] 15 at 30°C[a]	3.88[a,b,c] 3.9[d]
Dichlorobenzene		
1,2-	1 at 20°C[a] 1.28 at 25°C[e] 1.5 at 25°C[a] 1.9 at 30°C[a]	5.05[b] 5.07[a,c]
1,3-	1 at 12.1°C[b] 1.89 at 25°C[d]	5.08[b]
1,4-	0.6 at 20°C[a] 1.0 at 25°C[f] 1.8 at 30°C[a]	5.07[c] 5.08[b]
Trichlorobenzene		
1,2,3-	0.07 at 25°C[d] 1 at 40°C[b]	6.26[b]
1,2,4-	0.29 at 25°C[d] 1 at 38.4°C[b]	6.26[b]
1,3,5-	0.15 at 25°C[d] 10 mm at 78°C[b]	6.26[b]
Tetrachlorobenzene		
1,2,3,4-	1 at 68.5°C[g] 0.04 at 25°C[h]	NA
1,2,3,5-	1 at 58.2°C[g] 0.07 at 25°C[h]	NA
1,2,4,5-	0.05 at 25°C[i] 0.05 at 25°C[h]	7.4[b]

TABLE 3-3 (cont.)

Chemical	Vapor Pressure (mm Hg)	Specific Vapor Density (air = 1)
Pentachlorobenzene	1 at 98.6°C[g]	NA
Hexachlorobenzene	1 at 114°C[g] 1.68×10^{-5} at 25°C[j] 1.089×10^{-5} at 20°C[k]	9.84[a]

[a] Verschueren, 1977

[b] Sax, 1979

[c] Lowenheim and Moran, 1975

[d] NLM, 1982a

[e] Richardson, 1968

[f] Martin and Worthing, 1977

[g] Weast, 1980

[h] MacKay et al., 1982

[i] Ware and West, 1977

[j] Leoni and Darca, 1976

[k] Farmer et al., 1980

NA = Not available

very alkaline conditions. A description of each of the chlorinated benzenes follows.

Monochlorobenzene, which is the most polar of the chlorinated benzenes, is a colorless, volatile liquid with a pleasant almond-like odor that is classified as a flammable liquid by the U.S. Department of Transportation (NLM, 1982a). Monochlorobenzene is soluble in water to the extent of 499±8 mg/ℓ at between 20 and 30°C (Verschueren, 1977). It is miscible in all proportions in ethyl alcohol and diethyl ether, and is very soluble in carbon disulfide and benzene (NLM, 1982a). No established trade specifications exist for monochlorobenzene. Kao and Pottenberger (1979) reported two impurities for a typical analysis of monochlorobenzene: dichlorobenzenes at <0.1 wt percent and benzene at <0.05 wt percent. This implied a purity of 99.8% or higher for the sample. A product data sheet (Dow Chemical Company, 1977) listed a 99.9% purity for monochlorobenzene, while Allied Chemical Corporation (1973) stated a purity of 99.0% for its product (U.S. EPA, 1980a)

1,2-Dichlorobenzene is a clear, volatile liquid with a pleasant odor (NLM, 1980) and is combustible at room temperature. It has a solubility of 145 mg/ℓ in water at 25°C (Verschueren, 1977). 1,2-Dichlorobenzene is miscible with alcohol, ether, benzene, carbon tetrachloride, and acetone (NLM, 1980). The lack of industry-wide standards of purity for this chlorinated benzene is illustrated by the compositions reported for 1,2-dichlorobenzene by different sources shown in Table 3-4.

1,3-Dichlorobenzene is a colorless liquid that is combustible at room temperature. It can react violently with aluminum (NLM, 1981a). It has a solubility of 123 mg/ℓ in water at 25°C (Verschueren, 1977). 1,3-Dichlorobenzene is soluble in alcohol, ether and benzene, and is miscible with acetone, carbon tetrachloride and petroleum ether (NLM, 1981a).

TABLE 3-4

Reported Composition of Commercial 1,2-Dichlorobenzene

Constituent	Composition (%)					
	Standard Grade[a]	Standard Grade[b]	Mechanical Grade[c]	High Purity Grade[c]	Technical Grade[d]	Purified Grade[d]
C_6H_5Cl	NA	0.07	NA	NA	<0.05	<0.05
1,2-$C_6H_4Cl_2$	80	82.7	75-85	99.0	80.0	98.0
1,3-$C_6H_4Cl_2$	2	0.5	0.5	"balance"	<19.0	NA
1,4-$C_6H_4Cl_2$	17	15.4	15-25	NA	NA	NA
C_6H_3Cl (all isomers)	NA	1.6	NA	NA	<1.0	NA
1,2,4-$C_6H_3Cl_3$	NA	NA	NA	NA	NA	<0.2

[a] Dow Chemical Company, 1977

[b] Allied Chemical Company, 1973

[c] MCA, 1974

[d] Kao and Poffenberger, 1979

NA = Not available

1,4-Dichlorobenzene is a combustible crystalline solid that tends to sublime at ordinary temperatures. It possesses a distinctive odor that is noticeable at concentrations between 30 and 60 ppm (NLM, 1981a). It has a solubility of 79 mg/ℓ in water at 25°C (Verschueren, 1977). It is soluble at 25°C in ether, chloroform, carbon disulfide and benzene, and is miscible with alcohol and acetone (NLM, 1981b). The commercially available technical grade 1,4-dichlorobenzene may contain <0.5 wt percent of the other two isomers and also may contain <0.1 wt percent of monochlorobenzene and trichlorobenzene (Kao and Poffenberger, 1979). A product data sheet (Dow Chemical Company, 1977) stated a purity of 99.95% for that company's 1,4-dichlorobenzene. Product information from Montrose Chemical (1972) described a mixture of 35% 1,2-dichlorobenzene and 65% 1,4-dichlorobenzene (U.S. EPA, 1980a).

1,2,3-Trichlorobenzene is a white crystalline solid (platelets from alcohol) that is volatile with steam. It is slightly soluble (31.5 mg/ℓ) at 25°C in water, slightly soluble in alcohol, soluble in benzene and carbon disulfide, and very soluble in ether (NLM, 1981e; Yalkowsky and Valvani, 1980).

1,2,4-Trichlorobenzene is a colorless liquid at 25°C but may also take the form of rhombic crystals because of its low melting point of 16.95°C. It possesses a distinctive odor, similar to that of 1,4-dichlorobenzene, and is considered volatile with steam (NLM, 1981f). It is slightly soluble in water, 34.6 mg/ℓ at 25°C (Yalkowsky and Valvani, 1980); miscible with benzene, petroleum ether and carbon disulfide; slightly soluble in ethanol; and very soluble in diethyl ether (NLM, 1981f). An information sheet (Dow Chemical Company, 1977) listed a purity of 100% for its product. Kao and Poffenberger (1979) reported that commercial 1,2,4-trichlorobenzene may contain monochlorobenzene (<0.1 wt percent) and di- and tetrachlorobenzenes (<0.5 wt percent and <0.5 wt percent) with the 1,2,4-trichlorobenzene content being around 97%.

1,3,5-Trichlorobenzene takes the physical form of white crystals or needles. It is very slightly soluble (6.6 mg/ℓ at 25°C) in water; sparingly soluble in alcohol; and soluble in ether, benzene, petroleum ether, carbon disulfide and glacial acetic acid (NLM, 1982c; Yalkowsky and Valvani, 1980).

1,2,3,4-Tetrachlorobenzene is a white crystalline solid that appears as needles from alcohol (NLM, 1981c). It is very slightly soluble in water (4.3 mg/ℓ at 25°C); slightly soluble in alcohol; soluble in hot alcohol; and very soluble in ether, carbon disulfide, acetic acid and petroleum ether (NLM, 1981c; Yalkowsky and Valvani, 1980).

1,2,3,5-Tetrachlorobenzene is a solid that appears in the form of needles or white flakes. It is very slightly soluble in water (3.5 mg/ℓ at 25°C), slightly soluble in alcohol, and very soluble in carbon disulfide and petroleum ether (NLM, 1981d; Yalkowsky and Valvani, 1980).

1,2,4,5-Tetrachlorobenzene appears as white flakes or needles. It takes the form of monoclinic prisms from ether, alcohol or benzene. It is practically insoluble in water (0.6 mg/ℓ at 25°C), slightly soluble in hot alcohol, and soluble in ether, chloroform and carbon disulfide (NLM, 1982b; Yalkowsky and Valvani, 1980). A commercial 1,2,4,5-tetrachlorobenzene was analyzed as 97.0% pure; impurities were not identified (Kao and Pottenberger, 1979; Dow Chemical Company, 1977).

Pentachlorobenzene is a needle-like solid (NLM, 1979b). It is slightly soluble in water (0.56 mg/ℓ at 25°C); slightly soluble in ether, benzene and chloroform; and soluble in hot alcohol and carbon disulfide (NLM, 1979b; Yalkowsky and Valvani, 1980).

Hexachlorobenzene is a colorless crystalline (monoclinic prisms) solid. Its water solubility was reported as 0.005 mg/ℓ at 25°C (Yalkowsky and Valvani, 1980). Hexachlorobenzene is sparingly soluble in cold alcohol and soluble in benzene, chloroform and ether (NLM, 1979a). Impure commercial preparations may contain pentachlorobenzene (10-81,000 ppm), octachlorodibenzo-p-dioxin (0.05-212 ppm) and octachlorodibenzofuran (0.35-58.3 ppm) (Villeneuve et al., 1974).

According to the CRC Atlas of Spectral Data and Physical Constants for Organic Compounds (Grasselli, 1973) the following group trends in spectroscopic properties can be seen:

> There is a red shift in ultraviolet λ_{max} for the aromatic π to π^* transition with increasing chlorination (245 to 272 nm for monochlorobenzene; 291 to 301 nm for hexachlorobenzene). This implies that the more chlorinated the chlorinated benzene, the more likely is photodegradation at sea level by sunlight. Diagnostic infrared absorptions for all the chlorinated benzenes occur around 3.2-3.3 and 6.2-6.4 μm. All the proton NMR aromatic signals for chlorinated benzenes in carbon tetrachloride or deuterated chloroform solvents occur between 6.9 to 7.5 ppm with respect to tetramethylsilane. The mass spectra for all compounds are characterized by very intense molecular ions (M) (100% for all compounds except for pentachlorobenzene), and intense M-35 peaks. Thus, specific ion monitoring using the molecular ions and M-35 peaks is possible, increasing the sensitivity of analysis.
>
> The atmospheric chemistry of chlorobenzenes has been studied under laboratory conditions. Dilling et al. (1976) studied the photocatalzyed degradation of monochlorobenzene in an atmosphere containing 5 ppm nitric oxide and reported its half-life to be 8.7 hours under strong light at room temperature. Kanno and Nojima (1979) irradiated monochlorobenzene with light from a xenon lamp in the presence of nitric oxide and air and found the products to be chlorinated nitrobenzenes and nitrophenols. Uyeta et al. (1976) found that irradiation of several chlorobenzenes with natural sunlight for periods up to 56 days yielded polychlorinated biphenyls. Whether PCBs are formed under atmospheric conditions is unknown, but unlikely because of the low concentration. Yanagihari et al. (1977) studied the degradation of monochlorobenzene in a smog chamber (2 ppm chlorobenzene, 1 ppm NO_x) and found 7.5% degradation in 5 hours. Using higher concentrations (5000 ppm chlorobenzene and 1000 ppm NO), Kanno and Nojima (1979) found similar rates of degradation and identified one chloronitrobenzene and three chloronitrophenols as products. Rates of reaction of chlorobenzene with hydroxyl radical (Anbar and Neta, 1967) and singlet oxygen

(Graedel, 1978) are also available which allows half-life estimations of 0.5 and 2.6 years, respectively. Yanohihara et al. (1977) also studied the atmospheric photochemistry of o-dichlorobenzene and found 21.5% degradation in 5 hours and Nojima and Kanno (1980) found 7.6% of p-dichlorobenzene (high concentrations of test chemical and NO) in 5 hours of irradiation.

One study has examined the possibility of photocatalyzed degradation of the chlorinated benzenes. Oliver et al. (1979) exposed 1.4 dichlorobenzene in saturated aqueous solutions with various suspended sediments (titanium oxide, clays and samples of river sediments) to ultraviolet light. Degradation of the dichlorobenzene occurred only in the titanium oxide solution, possibly because of a shielding of the chemical from the light by the other sediments or a catalytic effect of titanium oxide, and did not occur in natural sediment systems. Korte et al. (1978) and Hustert et al. (1981) demonstrated that hexachlorobenzene was photochemically stable. The Hustert et al. (1981) study consisted of sunlight irradiation of an aqueous solution.

3.3. ANALYTICAL METHODOLOGY

The usual sampling and analytical methods for airborne chlorobenzenes involve the adsorption and concentration of airborne vapors on sorbentpacked cartridges followed by thermal desorption and gas chromatographic (GC) analysis using either flame ionization detection, electron capture detection, or photoionization detection. The purge and trap method is the most common method used for the sampling of volatile chlorobenzenes in water. Headspace analysis using GC with flame ionization detection or electrolytic conductivity detection are also used for analysis of aqueous samples. Methods that are slightly modified from the analytical procedures for aquatic samples are used for the analysis of chlorobenzenes in soil, food and human tissues. The following sections provide examples of these analytical methods.

3.3.1. Analysis in Air. Krost et al. (1982) described a method whereby ambient air was drawn through a cartridge containing a 1.5x6.0 cm bed of Tenax-GC (35/60 mesh) so that vapors were collected completely on the resin. The sample was then thermally desorbed and the vapors passed through a cryogenically cooled trap and subsequently introduced into a gas chromatograph-mass spectrometer (GC-MS). Estimated detection limits for three chlorobenzenes were as follows: monochlorobenzene, 2.1 ng/m³; 1,2-dichlorobenzene, 1.0 ng/m³; and 1,3-dichlorobenzene, 0.7 ng/m³. However, the precision of this method for field sampling and analysis has been determined to range from ±10 to ±40% relative standard deviation. A similar method has been used for the monitoring of mono- and dichlorobenzenes by Barkley et al. (1980), Pellizzari (1982) and Bozzelli (1981).

Lewis and MacLeod (1982) have developed and evaluated a portable low-volume air sampling system for indoor air monitoring of semivolatile organic chemicals. Two types of sampling cartridges were used to sample for chlorobenzenes. Using polyurethane foam (PUF), a mean collection efficiency of 94.0% with a relative standard deviation of 12% was determined for five 1 μg samples of pentachlorobenzene. For five 0.5 and 1.0 μg samples of hexachlorobenzene, the reported mean collection efficiency was 94.5% with a relative standard deviation of 8%. The tri- and tetrachlorobenzenes were poorly trapped using this PUF plug, with collection efficiencies of 6.6 and 62.3%, respectively. However, using a dual-sorbent trap consisting of a 0.6 g layer of Tenax-GC (35-60 mesh) sandwiched between two 3.8 cm PUF plugs, a collection efficiency of 98% was obtained for both compounds. Theoretical detection limits, using GC-electron capture detection, are expected to be at least one order of magnitude lower (in the range of 0.06-0.1 μg/m³). Storage stability of the PUF cartridges was tested under adverse storage conditions. The amount of chlorobenzenes recovered from the cartridges after 15 days of storage at 32°C ranged from 57% for the trichlorobenzenes to 98% for hexachlorobenzene. Billings and Bidleman (1980) reported that hexachlorobenzene was very poorly retained by porous

PUF, but efficiently collected by Tenax-GC. Oehme and Stray (1982), however, reported high recovery (76-115%) of tri-, tetra-, penta- and hexachlorobenzene with PUF plugs.

Langhorst and Nestrick (1979) used an air sampling tube packed with two sections of Amberlite XAD-2 resin separated by a silanized glass wool plug to collect the chlorobenzenes. The adsorbent was desorbed with carbon tetrachloride and analyzed by GC using a photoionization detector. Using the method described, the minimum detection limits for mono-, di-, tri-, tetra-, penta- and hexachlorobenzene were 15, 20, 30, 35, 45 and 70 ppb (v/v), respectively. Collection and desorption efficiencies for all chlorobenzenes (air concentrations between 5 ppb and 15 ppm) were ~95% with a precision of ±12%.

Thompson et al. (1980) described a sampling technique using the Trace Atmospheric Gas Analyzer coupled with negative atmospheric pressure chemical ionization for analysis of hexachlorobenzene after gas chromatography. This system is portable with close to real-time capability and a detection limit of ~30 ppt (v/v) in air.

Van Tassel et al. (1980) pointed out that there are disadvantages in using some sorbent materials because of interferences, such as relative humidity and high concentrations of carbon dioxide. They described a method for determining microgram-per-cubic meter levels of monochlorobenzene in air using sampling cartridges containing Porapak N, followed by elution with methanol and analysis by GC. Both electron capture detection and photoionization detection can be used with detection limits of 1 $\mu g/m^3$ and 5 $\mu g/m^3$, respectively. This technique reportedly allows for greater flexibility. Results are reproducible at various relative humidity levels and varying concentrations of carbon dioxide. Advantages over thermal desorption techniques include ease of operation and the ability to use various columns to achieve analytical precision.

NIOSH (1977) has developed sampling and analytical methods for occupational air monitoring for monochlorobenzene, 1,2-dichlorobenzene and 1,4-dichlorobenzene. All three methods involve trapping the compound in a charcoal tube, desorbing the analyte with carbon disulfide, and analyzing the sample in a gas chromatograph using flame ionization detection.

3.3.2. Chemical Analysis in Water. The purge-trap method is the most commonly used method for analyzing volatile organics in water. Otson and Williams (1982) evaluated the use of the dynamic headspace or the purge-trap method in combination with GC technique for a number of organics including monochlorobenzene and dichlorobenzene. For monochlorobenzene, a recovery rate of 91% was measured using flame ionization detection and 96% was measured using electrolytic conductivity detection. The corresponding recovery rates for 1,4-dichlorobenzene were 65 and 58%. Detection limits ranged from <0.1 μg/ℓ for monochlorobenzene to 0.2 μg/ℓ for 1,2-dichlorobenzene. The relative standard deviation ranged from 6.3-9.6% using flame ionization detection. Storage of samples for several weeks did not affect results by more than ±15%. The dynamic headspace or the purge-trap method has been used by other researchers to determine the levels of mono- and dichlorobenzenes in water samples (Pereira and Hughes, 1980; Jungclaus et al., 1978). The purge-trap technique is also recommended by U.S. EPA Method 602 (1982) for the determination of mono- and dichlorobenzenes in wastewater. Cowen and Baynes (1980) concluded that headspace analysis was applicable for monochlorobenzene and dichlorobenzenes, using flame ionization detection at the 5 μg/ℓ concentration in water. Minimum detectable quantities using electrolytic conductivity detection were 0.15 and 0.20 ng for monochlorobenzene and dichlorobenzenes, respectively. The static headspace technique was employed by Otson et al. (1982) for the quantification of mono- and dichlorobenzene in Canadian potable waters.

The purge-trap technique does not provide quantitative recoveries for compounds with low volatilities, such as trichlorobenzenes and higher chlorinated benzenes. Therefore, a solvent extraction and cleanup method is normally used to produce organic extracts suitable for GC/MS analysis. The U.S. EPA (1982) (Method 612) has recommended the use of Florisil column chromatography as a cleanup step before the quantification of the samples by GC with electron capture detector. This recommended method is applicable for the determination of di-, tri-, tetra-, penta- and hexa-chlorobenzene in drinking water and wastewater. The recovery of dichlorobenzenes and hexachlorobenzene by this method was found to be 82-89% and 95%, respectively. The percent standard deviation of the method for dichlorobenzenes and hexachlorobenzene ranged from 10-20% (U.S. EPA, 1982).

3.3.3. Chemical Analysis in Soil, Sediment and Chemical Waste Disposal Site Samples. A method for the determination of hexachlorobenzene in soil and chemical waste disposal site samples has been developed by DeLeon et al. (1980). The procedure involves methane extraction followed by temperature-programmed GC analysis using electron capture detection. Recoveries of samples spiked at the 10, 100 and 300 μg levels were 96.5% (±3.6), 93.1% (±8.1) and 78.0% (±2.6), respectively. The lower detection limit for this method is around 10 μg/g. The solvent extraction method was used by Lopez-Avila (1983) to determine chlorobenzenes in sediment samples. In this method, the solvent extract was subjected to acid-base fractionation. The base/neutral fraction containing the chlorobenzenes was fractionated by silica gel chromatography. The final separation and quantification was accomplished by GC-MS. The recovery of 1,3-dichlorobenzene, 1,2-dichlorobenzene, 1,2,4-trichlorobenzene and hexachlorobenzene by this method was 63, 66, 67 and 46%, respectively, at a spike level of 400 ng/g of dry sediment.

3.3.4. Chemical Analysis in Fish and Other Foods.

3.3.4.1. FISH -- The levels of pentachlorobenzene and hexachlorobenzene have been determined in fish samples using solvent extraction, solvent and sulfuric acid partitioning and GC with electron capture detection (Lunde and Ofstad, 1976).

Hiatt (1981) compared three analytical methods used to quantify monochlorobenzene levels in fish tissue. His data indicate that recoveries of 64±15%, 32±8%, and 68% were reported for the vacuum extraction method, direct purge and trap method, and a modified purge and trap method, respectively. In the modified purge and trap procedure, the homogenized fish tissue was purged, with the concentration trap immersed in an ice water bath for 5 minutes, followed by immersion in a 55°C water bath for an additional 7 minutes. This modification provided reproducible purging efficiencies. A similar modified purge-trap method has been used by Easley et al. (1981) for the determination of monochlorobenzene in fish samples.

The determination of trichlorobenzenes and other higher chlorinated benzenes in fish samples can also be done by a solvent extraction method. In one method, Kuehl et al. (1980) subjected the solvent extract to Florisil and gel permeation on chromatographic separation, followed by GC-MS identification and quantification of trichlorobenzene and other higher chlorinated benzenes in fish samples. Murray et al. (1980), however, used solvent partitioning, silica gel chromatography, followed by GC-electron capture detection for the quantification of hexachlorobenzene in fish samples.

3.3.4.2. HUMAN MILK -- A method to detect ppb concentrations of hexachlorobenzene in human milk has been used by Brevick (1978). This method involves solvent extraction and GC analysis using electron capture detection. A mean recovery of 98.6±10.8% was reported for 10 samples containing 5 ppb hexachlorobenzene. The solvent extraction method was also used by Tessari and Savage (1980) for the determination of hexachlorobenzene in human milk. In this method, the extract was subjected to Florisil and silica gel column chromatographic cleanup, followed by GC-electron capture detection. The method gave 68% recovery at a fortification level of 5.7 ng/g.

The quantification of more volatile halogenated benzenes, such as mono and dichlorobenzenes in milk samples, was performed by a purge and trap technique at an elevated temperature of 50°C. The trapped gas was thermally desorbed and quantified by the GC-MS method (Michael et al., 1980). The average recovery of monochlorobenzene by this method was determined to be 88%.

3.3.4.3. OTHER FOODS -- Rice, vegetables, meat, milk, eggs and fish have been analyzed for hexachlorobenzene residues using GC with electron capture detection (Sekita et al., 1980); GC-MS was used to confirm the analysis. A similar solvent extraction method, followed by solvent partitioning and Florisil column cleanup, and GC-electron capture detection was used for the quantification of hexachlorobenzene in different crops from 37 states (Carey et al., 1979).

3.3.4.4. OTHER BIOLOGICAL MATRICES -- Gas chromatography using electron capture detection has been employed to determine levels of pentachlorobenzene and hexachlorobenzene in blood samples (Lunde and Bjorseth, 1977) and to determine levels of 1,4-dichlorobenzene and its major metabolites in urine and serum samples (McKinney et al., 1970). Blood and urine samples have also been analyzed for the chlorobenzenes by GC using photoionization detection (Langhorst and Nestrick, 1979). Using carbon tetrachloride extraction, silica gel column chromatography and concentration with a Kuderna-Danish concentrator, chlorobenzene recoveries from blood and urine samples averaged 83±12% for concentrations between 1 and 500 ppb.

A method of hexachlorobenzene determination and confirmation in adipose tissue has been described by Watts et al. (1980). In this method, the solvent extract is subjected to a Florisil cleanup and one-fraction elution. Hexachlorobenzene is determined by direct GC with electron capture detection. Confirmation is made by analysis of the bis-isopropoxytetrachlorobenzene derivative, which is formed by reaction with isopropanol. Average recoveries ranged between 87.4±6.8% and 92.6±10.0%. This method is particularly useful for the determination of hexachlorobenzene in the presence of Mirex.

The determination of the less volatile chlorinated benzenes, such as tri-, tetra-, penta- and hexachlorobenzene in biological tissue samples, has been done by solvent extraction of the tissue, followed by column chromatographic cleanup of the sample and final separation and quantification by GC with electron capture detection (Lamparski et al., 1980; Mes et al., 1982). For more volatile chlorinated benzenes, such as mono- and dichlorobenzenes, the modified purge-trap method in combination with capillary GC and flame ionization detection or preferably more specific detection method can be used (Michael et al., 1980).

3.4. SUMMARY

The chlorinated benzenes are a group of compounds in which 1-6 chlorine substituents have been added to a benzene ring yielding a total of 12 isomeric forms. In general, these compounds have low water solubility (solubility decreasing with increasing chlorination), low flammability, moderate to high octanol/water partition coefficients (coefficients increasing with increasing chlorination) and low to moderate vapor pressures (vapor pressures decreasing with increasing chlorination). They are chemically unreactive and exist as liquids or solids at environmental conditions. Analysis of airborne chlorobenzenes is usually accomplished by adsorption onto sorbent cartridges, followed by thermal desorption and analysis by GC. For water samples, the purge-trap method is used to concentrate the volatile halogenated benzenes before analysis by GC. For less volatile chlorinated benzenes, solvent extraction followed by column chromatographic cleanup of the extract and GC with electron capture detection, is the most commonly used method for the isolation, detection and quantification. Methods similar to those used for wastewater samples are commonly used for the analysis of chlorinated benzenes in biological matrices.

4. PRODUCTION, USE AND ENVIRONMENTAL LEVELS

4.1. PRODUCTION

Industrial synthesis of chlorinated benzenes is achieved through the controlled catalytic chlorination of benzene and is described in the Kirk-Othmer Encyclopedia of Chemical Technology (Hardie, 1964). In general, monochlorobenzene and the dichlorobenzenes are synthesized at 30-50°C in the presence of a ferric chloride catalyst. The output is then purified by distillation, and the isomers are isolated by fractional distillation or crystallization. Trichlorobenzene is produced by the chlorination of dichlorobenzene using ferric chloride and temperatures of 25-30°C. An aluminum catalyst, trichlorobenzene and chlorine are used to produce tetrachlorobenzene, which in turn can serve as a precursor for pentachlorobenzene. Hexachlorobenzene can be obtained by the chlorination of benzene at 150-200°C using a ferric chloride catalyst or from the distillation of residues from the production of tetrachloroethylene. Because these reactions are not completely controlled and purification processes are not 100% effective, it is likely that any commercially available chlorinated benzene will also contain unwanted isomeric chlorobenzenes as impurities, and this is particularly true for 1,2-dichlorobenzene.

The TSCA Inventory (U.S. EPA, 1981) provides production data on the chlorinated benzenes for individual facilities. The data for the largest producers ($>1\times10^6$ pounds/year) are expressed as ranges of estimated production for 1977 in Table 4-1. Total yearly production data are published for high-volume, synthetic chemical intermediates by the U.S. International Trade Commission (USITC); data for 1980 are available only for monochlorobenzene, 1,2-dichlorobenzene and 1,4-dichlorobenzene (USITC, 1981), and are

TABLE 4-1

United States Production of Chlorinated Benzenes for Selected Years

Chemical/ Manufacturers	Location	Production Estimates for 1977[a] (lb x 10^6)	Production for 1980[b] (lb x 10^6)
Monochlorobenzene:			
Dow Chemical Co.	Midland, MI	50-100	
PPG Industries, Inc.	New Martinsville, WV	10-50	
Montrose Chemical Corp. of CA	Henderson, NV	10-50	
Allied Chemical Corp.	Solvay, NY	1-10	
Monsanto Co.	Sauget, IL	50-100[c]	
NA[d]	NA[d]	10-50	
NA[d]	NA[d]	1-10	
	Total:	132-370	284
1,2-Dichlorobenzene:			
Dow Chemical Co.	Midland, MI	1-10	
PPG Industries, Inc.	New Martinsville, WV	10-50	
Monsanto Co.	Sauget, IL	1-10	
Montrose Chemical Corp. of CA	Henderson, NV	1-10	
Allied Chemical Corp.	Solvay, NY	1-10	
	Total:	14-90	49
1,3-Dichlorobenzene:			
PPG Industries, Inc.	New Martinsville, WV	0.1-1	
NA[d]	NA[d]	0.1-1	
	Total:	0.2-2	NA

TABLE 4-1 (cont.)

Chemical/ Manufacturers	Location	Production Estimates for 1977[a] (lb x 10^6)	Production for 1980[b] (lb x 10^6)
1,4-Dichlorobenzene:			
Dow Chemical Co.	Midland, MI	1-10	
PPG Industries, Inc.	New Martinsville, WV	10-50	
Monsanto Co.	Sauget, IL	1-10	
Montrose Chemical Corp. of CA	Henderson, NW	1-10	
Allied Chemical Corp.	Solvay, NY	1-10	
Dover Chemical Corp.	Dover, OH	1-10	
NA[d]	NA[d]	1-10	
	Total:	16-110	75
1,2,3-Trichlorobenzene:			
Dow Chemical Co.	Midland, MI	1-10	
	Total:	1-10	NA
1,2,4-Trichlorobenzene:			
Dow Chemical Co.	Midland, MI	1-10	
NA[d]	NA[d]	10-50	
	Total:	11-60	NA
1,3,5-Trichlorobenzene:			
Chemical Systems Division	San Jose, CA	0.01-0.1	
	Total:	0.01-0.1	NA
1,2,3,4-Tetrachlorobenzene:			
Dow Chemical Co.	Midland, MI	1-10	
NA[d]	NA[d]	0.1-10	
	Total:	1.1-20	NA

TABLE 4-1 (cont.)

Chemical/ Manufacturers	Location	Production Estimates for 1977[a] (lb x 10^6)	Production for 1980[b] (lb x 10^6)
1,2,3,5-Tetrachlorobenzene: (No manufacturers listed)			
1,2,4,5-Tetrachlorobenzene: Dow Chemical Co.	Midland, MI	10-50	
Chem South Corp.	Childersburg, AL	0.1-1[c]	
NA[d]	NA[d]	1-10[c]	
	Total:	11.1-61	NA
Pentachlorobenzene: Olin Corp.	McIntosh, AL	1-10	
	Total:	1-10	NA
Hexachlorobenzene: (No manufacturers listed)			NA

[a] Source: U.S. EPA, 1981

[b] Source: U.S. ITC, 1981

[c] All production at this site was processed within the facility and was not distributed outside the facility as a chemical or in a mixture.

[d] Producer and location not listed in the TSCA inventory.

NA = Not available

incorporated into Table 4-1. A more recent list of producers and the estimates of their production capacities for chlorobenzenes are available from SRI (1983), who list the producers of chlorobenzenes and their estimated production capacities as of January 1983 (Table 4-2). The names of the chlorobenzene manufacturers given in Table 4-2 are slightly different from those given in Table 4-1, because Table 4-2 list only the manufacturers as of January, 1983.

As mentioned already, hexachlorobenzene is not manufactured commercially in the United States but does occur in waste streams during the production of some organic chemicals (e.g., perchloroethylene, trichloroethylene, carbon tetrachloride and chlorine) and pesticides.

4.2. USE

Chlorinated benzenes are used in manufacture of intermediates in the production of organic chemicals, including other chlorinated benzenes, herbicides, pesticides, dyes and rubber chemicals and as dye carriers, process solvents, pesticides, fungicides and deodorizing agents (U.S. EPA, 1980). A summary of these uses is presented in Table 4-3.

4.3. SOURCE AND ENVIRONMENTAL LEVELS

No comprehensive studies have been conducted on the sources of chlorobenzenes released into the environment. In general, these releases would occur during the manufacture and transport of chlorobenzenes, through their use as pesticides, solvents and other industrial and comsumer products, and through the disposal of wastes from the manufacturing process. Estimates of releases (Table 4-4) have been made for monochlorobenzene, dichlorobenzenes and trichlorobenzenes. Dow (1978) estimated that 30-50% of the monochlorobenzene produced annually was released into the air. Virtually all the monochlorobenzene used as a solvent in herbicide formulations is probably

TABLE 4-2

U.S. Producers and Estimated Annual Production Capacities (1983) of Chlorobenzenes*

Chemical/ Manufacturer	Location	Annual Capacity (lb. x 10^6)
Monochlorobenzene:		
Dow Chemical Co.	Midland, MI	170
Monsanto Co.	Sauget, IL	150
PPG Industries, Inc.	Natrium, WV	45
Standard Chlorine Chem.	Delaware City, DE	150
	Total:	515
1,2-Dichlorobenzene:		
Dow Chemical Co.	Midland, MI	30
Monsanto Co.	Sauget, IL	6
PPG Industries, Inc.	Natrium, WV	20
Standard Chlorine Chem.	Delaware City, DE	50
	Total:	106
1,3-Dichlorobenzene:		
NA	NA	NA
1,4-Dichlorobenzene:		
Dow Chemical Co.	Midland, MI	30
Monsanto Co.	Sauget, IL	12
PPG Industries, Inc.	Natrium, WV	30
Standard Chlorine Chem.	Delaware City, DE	75
	Total:	147
1,2,3-Trichlorobenzene:		
Standard Chlorine Chem.	Delaware City, DE	NA

TABLE 4-2 (cont.)

Chemical/ Manufacturer	Location	Annual Capacity ($lb. \times 10^6$)
1,2,4-Trichlorobenzene:		
Dow Chemical Co.	Midland, MI	NA
Standard Chlorine Chem.	Delaware City, DE	NA
1,3,5-Trichlorobenzene:		
Southland Corp.	Great Meadows, NJ	NA
Trichlorobenzene, Mixed Isomers:		
PPG Industries, Inc.	Natrium, WV	NA
1,2,3,4-Tetrachlorobenzene:		
NA	NA	NA
1,2,3,5-Tetrachlorobenzene:		
NA	NA	NA
1,2,4,5-Tetrachlorobenzene:		
Dow Chemical Co.	Midland, MI	NA
Standard Chlorine Chem.	Delaware City, DE	NA
Pentachlorobenzene:		
NA	NA	NA

*Source: SRI, 1983

NA = Not available

TABLE 4-3

A Summary of the Uses of the Chlorinated Benzenes

Chemical	Major Uses	Reference
Monochlorobenzene	Intermediate in the manufacture of chloronitrobenzenes, diphenyl oxide, DDT and silicones; as a process solvent for methylene diisocyanate, adhesives, polishes, waxes, pharmaceuticals and natural rubber; as a degrading solvent.	U.S. EPA, 1980
1,2-Dichlorobenzene	In the manufacture of 3,4-dichloroaniline; as a solvent for a wide range of organic materials and for oxides of non-ferrous metals; as a solvent carrier in production of toluene diisocyanate; in the manufacture of dyes; as a fumigant and insecticide; in degreasing hides and wool; in metal polishes; in industrial odor control; in cleaners for drains.	Hawley, 1977
1,3-Dichlorobenzene	As a fumigant and insecticide	Hawley, 1977
1,4-Dichlorobenzene	As a moth repellent, general insecticide, germicide, spare deodorant; in the manufacture of 2,5-dichloroaniline and dyes; as an intermediate; in pharmaceuticals; in agricultural fumigants.	Hawley, 1977
1,2,3-Trichlorobenzene	Other than chemical intermediate usage, the uses of this compound are the same as 1,2,4-trichlorobenzene.	U.S. EPA, 1980
1,2,4-Trichlorobenzene	As an intermediate in the manufacture of herbicides; as a dye carrier, as a dielectric fluid; as a solvent; as a heat-transfer medium.	U.S. EPA, 1980
1,3,5-Trichlorobenzene	Solvent for high-temperature melting products; as a coolant in electrical insulators; as a heat-transfer medium, lubricant and synthetic transformer oil; as a termite preparation and insecticide; in dyes.	Slimak et al., 1980
1,2,3,4-Tetrachlorobenzene	As a component in dielectric fluids; in the synthesis of fungicide.	Hawley, 1977
1,2,3,5-Tetrachlorobenzene	NA	U.S. EPA, 1980
1,2,4,5-Tetrachlorobenzene	As an intermediate for herbicides and defoliants; as an insecticide; moisture-resistant impregnant; in electric insulation; in packing protection.	Hawley, 1977
Pentachlorobenzene	In a pesticide used to combat oyster drills; as a chemical intermediate.	Clement Associates, 1979 Ware and West, 1977
Hexachlorobenzene	As a fungicide; industrial waste product in the manufacture of perchloroethylene, chlorinated solvents, pesticides and nitroso rubber.	Courtney, 1979

NA = Not available

TABLE 4-4

Estimated Quantities of Chlorobenzenes Lost During Manufacture, and to the Environment Compared with Total Production in 1983*
(All figures are in megagram)

Chlorobenzene	Quantity Lost During Manufacture	Quantity Lost to Environment	Total Industrial Production
Mono-	191-303	153-259	88,769-128,755
Di-			
1,2-	118-206	29.95	18,301-21,479
1,3-	0.185-0.608	NA	147-460
1,4-	178-284	166-269	28,310
Tri-			
1,2,3-	0.598-2.111	<0.100	23.3-74.1
1,2,4-	3.39-10.2	0.364-0.924	1,253-3,668
1,3,5-	import	import	111-210
Tetra-	NA	NA	NA
Penta-	not manufactured	NA	NA
Hexa-	not manufactured	NA	NA

*Source: 47 FR 26992

NA = Not available

released into the atmosphere. Approximately 0.1% of monochlorobenzene produced annually was estimated to enter water (Dow, 1978). The U.S. EPA (Gruber, 1975) estimated that, assuming all of the monochlorobenzene was produced using a batch process, <0.1% of its annual production would occur in wastewater and <1% would be disposed of on land. For 1,2-dichlorobenzene, Dow (1978) estimated that 5-10% of its annual production would be released into air and <0.1% into water. Using different data, U.S. EPA (1980) calculated that 2% of the annual production of the 1,2-isomer would be released into the environment during its manufacture. For 1,4-dichlorobenzene, which is used as a space deodorant, Dow (1978) stated that 70-90% of the yearly production would be released into air and <5% into water. U.S. EPA (1980) estimated that 2% of the total amount of 1,4-dichlorobenzene would be released during its production. Data were not available on potential environmental releases for other chlorinated benzenes.

Mumma and Lawless (1975) surveyed industrial processing data and identified nine products whose manufacture resulted in the generation of hexachlorobenzene. The authors estimated that 2.4-4.9 million pounds were generated in 1972 and that the manufacture of four products accounted for >95% of this total. These four products and the estimated quantities of hexachlorobenzene produced are listed in Table 4-5. Hexachlorobenzene is also a constituent of a seed treatment called Grannox NM that is imported and used in the United States. In 1975, ~200,000 pounds of Grannox NM, a formulation of hexachlorobenzene also containing Maneb, entered the United States (IARC, 1979). Table 4-4 shows the most recent official figures for quantities lost from industrial sources, and quantities released into the environment compared with total industrial production (47 FR 26992).

TABLE 4-5

Estimated Quantities of Hexachlorobenzene (HCB) in Industrial Wastes and Byproducts in 1972*

Product	Total HCB (10^3 lbs)	HCB (lbs/ton of product)
Perchloroethylene	1750-3500	4.8-9.5
Trichloroethylene	230-450	1.1-2.1
Carbon tetrachloride	200-400	0.4-0.8
Chlorine	160-390	0.02-0.04

*Source: Mumma and Lawless, 1975

4.3.1. Levels in Air. Investigations of the occurrence of chlorobenzenes in air have been conducted in Japan and the United States utilizing both grab and sorbent cartridge techniques. These studies, which have included the sampling of polluted air and urban and rural air, have reported the detection of monochlorobenzene, and various isomers of di-, tri-, tetra- and hexa-chlorobenzenes. Analysis of indoor air has indicated the presence of monochlorobenzene and the dichlorobenzenes, and other studies have measured monochloro- and 1,4-dichlorobenzene in occupational settings.

Morita and Ohi (1975) sampled ambient air for the determination of 1,4-dichlorobenzene levels at six central and suburban Tokyo sites in Japan and found concentrations ranging from 1.5-4.2 $\mu g/m^3$. Determinations were also performed on "indoor" samples from a closet, a bedroom and a wardrobe; these concentrations were ~25-400 times greater than the highest reported ambient levels.

Pellizzari et al. (1979) presented the results of the analysis of air samples collected from a number of locations in the United States. Samples from each location were obtained from several sites at a given location and at numerous times. The samples were analyzed for monochlorobenzene and the di- and trichlorobenzenes. Table 4-6 is a compilation of these data.

Monochlorobenzene and the dichlorobenzenes were also measured by Wojinski et al. (1979) in samples from an industrially produced cloud that periodically appears over Henderson, Nevada industrialized town 10 miles southeast of Las Vegas. Monochlorobenzene levels were nearly 50 times greater in the Henderson samples (mean: 24325 ng/m^3) as compared with the Las Vegas samples (mean: 458 ng/m^3). The mean 1,2-dichlorobenzene concentrations were ~5 times greater over Henderson than over Las Vegas (10291 ng/m^3 compared with 3087 ng/m^3). However since the methodology was different, the significance of this finding is uncertain.

TABLE 4-6

Chlorinated Benzene Levels in Ambient Air from Different Locations in the U.S.[a]

Site	Date Sampled	Concentration range, ng/m^3						
		MCB	1,2-DCB	1,3-DCB	1,4-DCB	TCB	TeCB	PeCB
Kin-Buc Disposal Site, Edison, NJ	1976/ 1978	ND-12,791	ND-12,433	ND-33,783	ND-7000	ND-1327	NR	NR
Baton Rouge, LA	1977	ND-900	ND-87	ND-102	ND	ND	NR	NR
Houston, TX	1977	ND-132	ND-86	ND-86	ND	ND	NR	NR
Niagara Falls, NY	NR	ND-119	ND-444[b]	ND-444[b]	ND-444[b]	ND-4346	ND-451	ND-17
Different NJ Sites[c]	1978	ND-6072	ND-5513	ND-3392	ND	ND	NR	NR

[a] Source: Taken from Pellizzari et al., 1979

[b] These are the values for the unseparated isomers

[c] The sites include: Edison, Ground Brook, Paterson, Hoboken, Clifton, Fords, Passaic and Sayreville

ND = Not detected; NR = not reported

MCB = Monochlorobenzene; DCB = dichlorobenzene; TCB = trichlorobenzene; TeCB = tetrachlorobenzene; PeCB = pentachlorobenzene

In a study of contamination from a hazardous waste site, Barkley et al. (1980) provided data on chlorobenzene levels from the homes of nine residents of the "Old Love Canal" area of Niagara Falls, New York. Monochlorobenzene was detected inside three of the homes at concentrations ranging from 60-600 ng/m³. The dichlorobenzenes (isomers unseparated) were found inside the homes at concentrations ranging from trace levels to 31,000 ng/m³. Samples taken outside the homes over 6-16 hour periods contained monochlorobenzene, dichlorobenzenes (isomers unseparated), trichlorobenzenes (isomers unseparated) and tetrachloro-benzenes (isomers unseparated) from nondetectable amounts up to 440 ng/m³, the highest level being found at one location for the dichlorobenzene isomers.

In 1978, the Department of Environmental Protection of New Jersey initiated a study of selected volatile organic chemicals in ambient air. Over a 5-month period, a total of 330 samples were obtained at five sites that included a mixture of industrial, residential and semirural locations. The results, reported by Bozzelli and Kebbekus (1979), indicated the presence of 1,2- and 1,4-dichlorobenzene at all sites. The average concentrations (trace amounts were averaged as the lower detection limit of 0.01 ppb) were 2096 ng/m³ and 1703 ng/m³ for 1,2- and 1,4-dichlorobenzene, respectively. Peak concentrations were 46,780 ng/m³ and 93,560 ng/m³, respectively. In a follow-up study, Harkov et al. (1981) sampled air at six sites in New Jersey over 24-hour periods every 6 days for a year. Monochlorobenzene was detected in 86% of the samples at an average level of 2.53 μg/m³ and a maximum level of 1.36 μg/m³.

Field studies were conducted by Singh et al. (1981) in California and Arizona to characterize the atmospheric levels and fate of several organic chemicals. The samples, collected over 24 hours during a 2-week period at each site, were analyzed for four chlorobenzenes: monochlorobenzene, 1,2-dichlorobenzene, 1,3-dichlorobenzene and 1,2,4-trichlorobenzene. Table 4-7 presents the results of the analysis.

The atmospheric concentrations of the chlorinated benzenes around different locations in the United States have been measured by a number of other investigators. The monitoring sites can be broadly divided into three different categories, namely rural/remote sites, urban/suburban sites and sites near source areas. In a recent report, Brodzinsky and Singh (1982) integrated most of the available U.S. air monitoring data relating to the levels of chlorobenzenes along with a number of other organics into a coherent data base. The overall and site-specific mean atmospheric levels of monochlorobenzene, dichlorobenzenes, trichlorobenzenes and tetrachlorobenzenes compiled in this report (Brodzinsky and Singh, 1982) are shown in Table 4-8.

The levels of pentachlorobenzene and hexachlorobenzene in ambient air samples rarely have been reported. Considering their volatility, the abundance of these compounds in the atmosphere can be speculated to be lower than the other chlorobenzenes. However, tetrachlorobenzenes, pentachlorobenzene and hexachlorobenzene have been detected, but not quantified, in fly ashes from municipal incinerators (Eiceman et al., 1979, 1981). High volume air samples collected from Boston, Massachussetts, and Columbia, South Carolina, using a glass fiber filter and polyurethane foam trap were subjected to interlaboratory analysis of hexachlorobenzene along with other parameters (Bidleman, 1981). The average concentrations of hexachlorobenzene in Boston (Massachussetts) and Columbia (South Carolina) air were found to be >0.057 ng/m^3 and 0.012 ng/m^3, respectively. The percent relative standard deviations for the two results were determined to be 35 and 43%, respectively.

TABLE 4-7

Concentrations of Chlorinated Benzenes at Three Sites[a]

Chemical	Mean Concentration in ng/m³ ± 1 Standard Deviation[b]		
	Los Angeles, CA	Phoenix, AZ	Oakland, CA
Monochlorobenzene	~936	~936	~468
1,2-Dichlorobenzene	75.1 ± 59.5	135.8 ± 209.1	24.0 ± 30.1
1,3-Dichlorobenzene	46.3 ± 33.7	52.3 ± 35.5	39.1 ± 17.4
1,2,4-Trichlorobenzene	52.0 ± 36.9	23.4 ± 15.8	22.6 ± 18.1

[a] Source: Singh et al., 1981

[b] The conversion of ppt unit to ng/m³ is based on a temperature of 20°C and a pressure of 1 atmosphere.

TABLE 4-8

Overall and Site-Specific Mean Atmospheric Levels of Chlorobenzenes throughout the United States*

Chemical	Total Number of Localities	Mean Concentration, ng/m^3			
		Overall	Rural-Remote Area	Urban-Suburban Areas	Areas of Production
Monochlorobenzene	56	3087	ND	3742	936
1,2-Dichlorobenzene	51	1142	11	1142	1202
1,3-Dichlorobenzene	38	571	40	499	902
1,4-Dichlorobenzene	24	1563	ND	1743	16
Trichlorobenzenes	35	136	ND	128	181
Tetrachlorobenzenes	3	3502	198	6196	853

*Source: Brodzinsky and Singh, 1983

ND = Not detected

In a survey of contamination by hexachlorobenzene around eight individual plants, Li et al. (1976) reported the detection of up to 24 $\mu g/m^3$ hexachlorobenzene at a distance of 90 feet from one plant. Table 4-9 summarizes the data from this investigation. Concentrations of hexachlorobenzene at distances 400-3000 feet downwind from the plants ranged from 0.02-2.7 $\mu g/m^3$. The authors noted that the highest levels of hexachlorobenzene contamination were associated with the production of lower chlorinated hydrocarbons as opposed to the production of chlorine and herbicides, and that plants with onsite landfill and open pit waste disposal sites had the highest levels of airborne concentrations of hexachlorobenzene.

Chlorinated benzenes are also present in air in occupational settings. Ware and West (1977) reported that the air of facilities manufacturing 1,4-dichlorobenzene contained an average of 204 mg/m^3 dichlorobenzene (from 42-288 mg/m^3) for certain processes and that no levels <48 mg/m^3 were detected throughout the plant. More recently, a survey of monochlorobenzene exposure was conducted at the chemical companies by the National Institute for Occupational Safety and Health (NIOSH) (Cohen et al., 1981). Personal sampling data indicated that monochlorobenzene levels ranged from below the detection level to 18.7 mg/m^3.

4.3.2. **Water**. Chlorinated benzenes have been detected in ground, surface and drinking water and in industrial and municipal wastewater. The most prevalent compound is monochlorobenzene. The dichlorobenzenes, trichlorobenzenes, tetrachlorobenzenes, pentachlorobenzene and hexachlorobenzene are detected infrequently.

Numerous investigations have identified chlorinated benzenes in samples of surface water (Table 4-10). The U.S. EPA STORET system also includes monitoring data on the chemicals.

TABLE 4-9

Atmospheric Levels of Hexachlorobenzene Around Selected Industrial Plants*

Company/Location	Products	Hexachlorobenzene Concentrations, µg/m³	
		High	Low
Vulcan Materials Wichita, KS	Perchloroethylene, carbon tetrachloride, chlorine	24	0.53
Stauffer Chemical Louisville, KY	Perchloroethylene, carbon tetrachloride, methylene chloride, chlorine	7	0.24
Dow Chemical Pittsburgh, CA	Carbon tetrachloride, perchloroethylene, chlorine	0.08	<0.02
Du Pont Corpus Christi, TX	Carbon tetrachloride	ND	ND
Diamond Shamrock Deer Park, TX	Trichloroethylene, perchloroethylene, chlorine	ND	ND
Olin McIntosh, AL	Pentachloronitrobenzene, chlorine	2.2	0.03
Ciba-Geigy St. Gabriel, LA	Atrazine, propazine, simazine	0.02	ND
PPG Lake Charles, LA	Trichloroethylene, perchloroethylene, vinyl chloride, vinylidene chloride, chlorine, etc.	1.7	ND

*Source: Li et al., 1976

ND = Not detected (<0.02 µg/m³)

TABLE 4-10

Chlorinated Benzenes in Surface Water

Chemicals	Levels[a]	Location	Reference
Trichlorobenzene[b]	100-500	Merrimack River, MA	Hites, 1973
Monochlorobenzene	+	Glatt River, Germany	Giger et al., 1976
1,4-Dichlorobenzene	30-900		
Monochlorobenzene	+	River Waters, U.S.	Shackelford and Keith, 1976
Dichlorobenzene			
Trichlorobenzene			
Tetrachlorobenzene			
Pentachlorobenzene			
Hexachlorobenzene			
Hexachlorobenzene	ND-17.4 (2.7)	Tiber River, Italy	Leoni and D'Arca, 1976
Monochlorobenzene	ND-7000	Delaware River	Sheldon and Hites, 1978
Dichlorobenzene[b]	ND-400		
Trichlorobenzene[b]	ND-1000		
Monochlorobenzene	ND->10,000	Ohio River	ORVWSC, 1982
1,4-Dichlorobenzene	ND->10,000		
Dichlorobenzene[b]	+ in 7% of all surface water and in 3% of all groundwater samples	600 sites in NJ	Page, 1981
Monochlorobenzene	+	Drainage streams Niagara Falls, NY	Elder et al., 1981
Dichlorobenzene	+		
Trichlorobenzene	100-8000		
Tetrachlorobenzene[b]	100-200,000		
Pentachlorobenzene	ND-100,000		
Hexachlorobenzene	8000-30,000		

TABLE 4-10 (cont.)

Chemicals	Levels[a]	Location	Reference
Dichlorobenzene[c]	3-71 (27)	Great Lakes	Oliver and Nichol, 1982
Trichlorobenzene[c]	0.1-1.6 (0.5)		
Tetrachlorobenzene[c]	ND-0.8 (0.12)		
Pentachlorobenzene[c]	ND-0.6 (0.12)		
Hexachlorobenzene	0.02-0.1 (0.05)		
Dichlorobenzene[c]	ND-77 (11)	Grand River, Canada	Oliver and Nichol, 1982
Trichlorobenzene[c]	ND-8.7 (2.1)		
Tetrachlorobenzene[c]	ND-0.2 (0.05)		
Pentachlorobenzene	ND-0.1 (0.05)		
Hexachlorobenzene	0.02-0.1 (0.06)		

[a] Range (mean) in ng/ℓ unless indicated

[b] Unidentified isomers

[c] All isomers

ND = Not detected; + = detected

Drinking water supplies also have been sampled for chlorobenzene contamination. In a survey of three water treatment plants of the city of New Orleans, Louisiana, U.S. EPA (1975a) reported finding only one chlorobenzene, 1,3-dichlorobenzene, at two of the plants. The reported concentration was <3 μg/ℓ. The U.S. EPA also found monochlorobenzene and all three isomers of dichlorobenzene at levels <1.0 μg/ℓ in three of five raw water supplies that were sampled as part of the National Organics Reconnaissance Survey (U.S. EPA, 1975b). A follow-up study (U.S. EPA, 1975c) reported concentrations of monochlorobenzene in samples of finished drinking water from the four initial locations and at five additional sites.

Coniglio et al. (1980) reported data on concentrations of volatile organic chemicals from drinking water treatment plants in the United States. The frequency of occurrence of 1,2-dichlorobenzene and 1,2,4-trichlorobenzene in finished water originating from surface water was 12.5 and 11.5%, respectively. Of the facilities using groundwater, 12.9 and 7.1% had drinking water samples containing 1,4-dichlorobenzene and monochlorobenzene, respectively. The other chlorobenzenes were detected in <4.5% of the samples. The authors also reported data from a groundwater survey conducted in New Jersey. Of the chlorobenzenes, only the dichloro- and trichlorobenzenes (isomers unspecified) were detected at concentrations ranging from <1-100 μg/ℓ in 1 and 3% of the samples, respectively.

Fielding et al. (1981) sampled untreated and finished groundwater, raw and finished river water and rainwater from 13 unspecified sites throughout Great Britain. 1,4-Dichlorobenzene was detected at a concentration of 0.08 μg/ℓ in one finished groundwater sample. Page (1981) also surveyed groundwater and surface water at over 1000 sites throughout New Jersey, in a study designed to compare the relative degree of chemical contamination of ground and surface water. All isomers of dichlorobenzene were found in ~3% of all the groundwater samples and 4% of the surface water samples. Analysis of these data and data on 52 other toxic chemicals indicated that New Jersey groundwater has a similar pattern and degree of contamination as surface water. Oliver and Nichol (1982) sampled drinking water in three cities on Lake Ontario, both before and after chlorination. Individual isomers of dichloro- through hexachlorobenzene were found in mean concentrations ranging from non-detectable to 13 ng/ℓ. No increase in the level of concentration was noted in these compounds after chlorination. The levels of chlorobenzenes in the drinking water of three cities bordering Lake Ontario are given in Table 4-11.

As part of a study of the contamination of drinking water by leachate from a pesticide waste dump site in Hardeman County, TN, Clark et al. (1980) reported data from a U.S. EPA survey of chemicals in private wells. Monochlorobenzene was found in 23 of 25 wells at levels from trace amounts to 41 μg/ℓ with a median value of 5.0 μg/ℓ. In another study of possible contamination of drinking water by the disposal of toxic chemicals, Barkley et al. (1981) found monochlorobenzene and dichlorobenzenes (isomers unspecified) in tapwater from all nine of the houses in Old Love Canal, Niagara Falls area. Concentration levels for these two compounds ranged from 10-60 ng/ℓ and from 10-800 ng/ℓ, respectively. The tetrachlorobenzenes and pentachlorobenzene also were found at concentrations up to 2000 and 240 ng/ℓ, respectively.

The chlorobenzenes have been identified in wastewaters from industrial processes and in influents and effluents at municipal sewage treatment plants. Gaffney (1976) sampled water at four municipal facilities in Georgia that, in addition to handling sewage, also treated wastewater from

TABLE 4-11

Chlorobenzene Concentrations in Drinking Water from Ontario, Canada[a]

Chemical	Concentration ng/ℓ	
	Range	Mean
1,2-Dichlorobenzene	ND-7	3
1,3-Dichlorobenzene	ND-2	1
1,4-Dichlorobenzene	8-20	13
1,2,3-Trichlorobenzene	0.1-0.1	0.1
1,2,4-Trichlorobenzene	1-4	2
1,3,5-Trichlorobenzene	ND[b]	ND[b]
1,2,3,4-Tetrachlorobenzene	0.1-0.4	0.3
1,2,3,5-Tetrachlorobenzene	ND[b]	ND[b]
1,2,4,5-Tetrachlorobenzene	ND[b]-0.3	0.2
Pentachlorobenzene	0.03-0.05	0.04
Hexachlorobenzene	0.06-0.2	0.1

[a] Source: Oliver and Nichol, 1982

[b] Limits of detection were ~0.1 ng/ℓ for the trichlorobenzenes and ~0.05 for the tetrachlorobenzenes.

ND = Not detected

local synthetic carpet mills. Average concentrations reported for dichlorobenzenes in the incoming and outgoing water ranged from 3-146 μg/ℓ and 0-268 μg/ℓ, respectively. For the trichlorobenzenes, the levels ranged from 1-60 μg/ℓ and 0-13 μg/ℓ for influent and effluent, respectively. The author concluded that the increase in the dichlorobenzene levels was a result of chlorination performed during the secondary phase of wastewater treatment.

A U.S. EPA survey of wastewater throughout the United States found that dichlorobenzenes and trichlorobenzenes occurred in discharges from industrial and municipal plants (Ware and West, 1977). The reported concentrations ranged from 15-690 μg/ℓ for 1,2-dichlorobenzene and from 0.25-500 μg/ℓ for 1,2,4-trichlorobenzene. Young and Hessan (1978) also reported the presence of several chlorobenzenes in the wastewater of major municipal facilities in southern California. The highest concentrations they found were for water discharged by the Los Angeles Hyperion Treatment Facility during December: 1,2-dichlorobenzene, 435 μg/ℓ; 1,4-dichlorobenzene, 230 μg/ℓ; 1,2,4-trichlorobenzene, 130 μg/ℓ and 1,3,5-trichlorobenzene <0.2 μg/ℓ. For the other sites, the levels of dichlorobenzene isomers ranged from 0.2-6 μg/ℓ. None of the facilities used chlorination to treat the water.

Neptune (1980) compiled data for organic priority pollutants analyzed in samples taken during several industrial wastewater surveys conducted from September 1978 through October 1979. The resulting data were grouped by their occurrence in each of 35 standard industrial categories. The most prevalent of the chlorobenzenes was monochlorobenzene, which was present in waste streams from 14 industrial categories; 1,2-, 1,3- and 1,4-dichlorobenzene were detected in 13, 10 and 14 categories, respectively, with

1,2,4-trichlorobenzene present in 7 categories. The frequency and range of concentrations found are summarized in Table 4-12.

4.3.3. Food. Investigation of the occurrence of chlorobenzenes in food has been limited primarily to the measurement of hexachlorobenzene. This concern derived from its use as a fungicide on the seeds of several food crops and from its ability to bioaccumulate in the food chain. The bioaccumulation potential of hexachlorobenzene and the other chlorobenzenes is discussed in Section 5.3.

Johnson and Manske (1976) reported the detection of hexachlorobenzene at levels of 0.0006-0.041 mg/kg in food samples from 30 U.S. cities obtained from the Total Diet Program of the U.S. Food and Drug Administration (FDA). Based on these and other data, FDA estimated the average daily intake of hexachlorobenzene from foods in fiscal years 1973 and 1974 to be 0.3978 and 0.0725 μg/day, respectively (IARC, 1979). Leoni and D'Arca (1976), using analysis data on cooked foods served in the Italian Navy, estimated an average daily intake of 4.11 μg of hexachlorobenzene. In addition, these authors surveyed uncooked foods available to the public and found mean hexachlorobenzene levels ranging from none detected to 133.0 ppb (0.133 mg/kg), with the highest levels occurring in butter, lard and pork meat. The average daily intake of hexachlorobenzene from uncooked diets was calculated to be 4.32 μg, a value similar to intake from cooked diets. Hexachlorobenzene has also been detected in Navy foodstuffs available in Japan (Morita et al., 1975a,b; Sekita et al., 1980) and was measured in beef (12 μg/kg), salmon (9 μg/kg), pork (7μg/kg) and other animal sources of protein (Morita et al., 1975a,b). In a survey of over 300 suppliers of cow's milk in southern Ontario in 1977, hexachlorobenzene residues were identified in 68% of the samples at a level of 0.002 mg/kg in fat. The hexachlorobenzene

TABLE 4-12

Frequency and Range of Concentrations of Chlorinated Benzenes Pollutants in Industrial Wastewaters*

Chemical	Total Samples	Samples Containing >10 μg/ℓ		
		Number of Samples	Range (μg/ℓ)	Mean Concentration (μg/ℓ)
Monochlorobenzene	31,194	147	11-6,400	667
1,2-Dichlorobenzene	3,268	80	12-860	141
1,3-Dichlorobenzene	3,268	44	10-39	21
1,4-Dichlorobenzene	3,268	88	10-410	79
1,2,4-Trichlorobenzene	3,266	30	12-607	161

*Source: Neptune, 1980

levels found in the 1977 survey were significantly below the levels detected in a similar survey conducted in 1973 (Frank et al., 1979).

Some information on the presence of other chlorobenzenes in food was also available. Morita et al. (1975c) reported detecting 1,4-dichlorobenzene in fish including mackerel caught in Japanese coastal waters. Oliver and Nichol (1982) reported detecting all isomers of dichlorobenzene, trichlorobenzene, tetrachlorobenzene, pentachlorobenzene and hexachlorobenzene in trout from the Great Lakes. The highest levels were detected for penta- and hexachlorobenzene and the mean concentrations were 5.5 and 46.8 μg/kg, respectively. Residues of pentachlorobenzene have also been found in oils, fats and shortening (0.001-0.11 mg/kg) and sugar (~0.002 mg/kg) (U.S. EPA, 1980).

4.3.4. Soil and Sediments. The study of soil contamination by chlorobenzenes has concentrated on hexachlorobenzene, although more recent surveys have included all the chlorobenzenes.

Hexachlorobenzene has been detected in sediment samples taken from lakes throughout Germany (Buchert et al., 1981) and was measured at 0.04 ppm in soil from a farming region in Italy where high mortality among birds had taken place (Leoni and D'Arca, 1976). In 1975, the U.S. EPA examined soil and aquatic sediments at 26 locations along a 150-mile transect of Louisiana and found that 46% of the soil samples were contaminated with hexachlorobenzene at levels of 20-440 ppb. The aquatic sediments contained hexachlorobenzene at levels of 40-850 ppb (Blackwood and Spies, 1979). In a survey of nine industrial plants (see Table 4-9 for site locations) producing chlorocarbon compounds, Li et al. (1976) detected hexachlorobenzene in soil samples taken within the plant area at levels >1000 μg/g (1000 ppm) at three plants. Soil taken from the cornfield adjacent to one plant contained

1.1 μg/g (1100 ppb) and >3000 μg/g were detected along a boundary road of another plant.

Elder et al. (1981) sampled sediments in streams draining the Love Canal area of Niagara Falls, NY, and detected all of the chlorobenzenes (isomers unspecified) at levels in the ppm range. Oliver and Nichol (1982) studied the fate and distribution of chlorobenzenes in the Great Lakes and reported detecting all isomers in the sediments of Lakes Superior, Huron, Erie and Ontario. The most contaminated lake was Lake Ontario, for which the mean levels in the sediments of the individual isomers ranged from 11-94 ng/g for the dichlorobenzenes, from 7-94 ng/g for the trichlorobenzenes, and from 6-52 ng/g for the tetrachlorobenzenes. The levels of penta- and hexachlorobenzene were measured as 32 and 97 ng/g, respectively.

4.3.5. Human Tissue Residues. Studies of the transport, fate and bioaccumulation of the chlorinated benzenes reviewed above indicate that human exposure is likely from air, food and drinking water (Sections 5.1., 5.2. and 5.3.). In this section, human ambient exposure is confirmed by the reported levels of chlorobenzene in human adipose tissue, blood, breath and urine; unfortunately, the environmental concentrations were not available for comparison with the observed tissue concentrations.

Due to the lipophilic character of the chlorinated benzenes, as indicated by their octanol/water partition coefficient discussed in Section 5.3., adipose and other fatty tissues are the major tissue depots for chlorinated benzenes. The measured levels of several chlorobenzene isomers in human adipose tissue are shown in Table 4-13.

Human breast milk is also high in fat content, making this an exposure route for nursing infants. Stacy and Thomas (1975) analyzed breast milk

TABLE 4-13

Chlorinated Benzene Residues in Human Adipose Tissue

Compound	Country	Tissue Concentration (mg/kg)*	Reference
1,4-Dichlorobenzene	Japan	2.3	Morita and Ohi, 1975
	Japan	1.88	Morita and Ohi, 1975
	Japan	1.7	Morita et al., 1975?
1,2,4,5-Tetrachlorobenzene	Japan	0.019	Morita et al., 1975?
Hexachlorobenzene	Japan	0.21	Morita et al., 1975?
	United States	0.03-0.47	Barquet et al., 1981
	Italy	0.491	Leoni and D'Arca, 1976
	Great Britian	0.05	Abbott et al., 1972
	Germany	6.3	Acker and Shulte, 1970
	New Zealand	0.31	Solly and Shanks, 1974
	Canada	0.001-0.52	Mes et al., 1979
	Canada	0.01-0.67	Mes et al., 1982
	Sweden	0.029-0.071	Noren, 1983

*Values are for adipose tissue

samples from 20 urban and 20 rural Australian mothers and found the concentration of hexachlorobenzene in rural milk (0.079 mg/kg milk) significantly greater than that in urban milk (0.028 mg/kg). A study of another group of Australian mothers showed the opposite results: rural milk contained 0.042 mg/kg while urban milk contained 0.063 mg/kg (Newton and Greene, 1972). In France, 18 of 49 breast milk samples contained hexachlorobenzene at concentrations of 0.001-0.17 mg/kg whole milk (0.50-3.50 mg/kg on fat basis) (Goursaud et al., 1972). Relatively low concentrations of pentachlorobenzene and hexachlorobenzene (0.002 mg/kg and 0.006 mg/kg, respectively) were found in milk samples from Yugoslavian women (Kodric-Smit et al., 1980). In another study, 50 milk samples from Helsinki women in 1982 (Wickstrom et al., 1983) contained 0.7-6 μg hexachlorobenzene/kg whole milk (14-240 μg hexachlorobenzene/kg milk fat). No detectable hexachlorobenzene was found, however, in 57 samples of breast milk from women of rural Arkansas and Mississippi (Strassman and Kutz, 1977). Levels in two Swedish women varied from 0.029±0.002 mg hexachlorobenzene/kg milk fat in one to 0.071±0.005 mg hexachlorobenzene/kg milk fat in the other (Noren, 1983). Courtney (1979) reviewed some of these and other studies that substantiate the ubiquity of hexachlorobenzene by the fact that people with no known exposure to the chlorobenzene had measurable tissue concentrations.

In a study involving 28,000 people across the United States (Murphy et al., 1983), hexachlorobenzene was found in 4% of 4200 blood serum samples using a method with a detection limit between 1 and 2 μg/ℓ. In addition, hexachlorobenzene was found in 93% of 785 adipose tissue samples, using a method with detection limits around 10-20 μg/ℓ. These findings were interpreted as signifying non-occupational exposures. No actual levels were provided in this study.

From the data on chlorobenzene concentrations found in human blood and plasma, it is apparent that newborns to adults, even those from industrially remote areas or those with no known chlorobenzene exposure, experience exposures to these compounds.

Astolfi et al. (1974) reported hexachlorobenzene at 19 ng/ℓ in the umbilical cord blood of infants born in Argentina. Ninety-seven rural and 97 urban children from Upper Bavaria all had detectable levels of hexachlorobenzene in their blood ranging from 2.8-77.9 ppb (ng/g); the average concentration was 16.5 ppb (Richter and Schmid, 1976). An average concentration of 22 ppb hexachlorobenzene was measured in the blood of nonexposed Australians, whereas occupationally exposed people had an average concentration of 55.5 ppb (range 21-100 ppb) in their blood (Siyali, 1972). Morita and Ohi (1975) analyzed the blood of four male and two female residents of Tokyo for 1,4-dichlorobenzene and reported an average of 9.5 μg/ℓ.

Wastes containing hexachlorobenzene were spread on a landfill in western Louisiana as a fly control measure (Burns and Miller, 1975). Blood levels of hexachlorobenzene in 22 husband-wife pairs living near the landfill were analyzed. The average blood level for the men was 5.10 ppb, which was significantly higher than that for the women, which was 1.70 ppb. Forty-six Louisiana residents not living in the immediate vicinity of the landfill had average blood levels of 0.5 ppb hexachlorobenzene, while chemical plant workers in the area had a blood concentration range of 14-233 ppb. The levels of chlorinated benzenes in the blood of nine residents of the Love Canal area in Niagara Falls, New York, were measured and are shown in Table 4-14 (Barkley et al., 1980).

Although the chlorobenzenes bioaccumulate in human adipose tissue and are detected in human blood, the levels are tempered by the elimination processes. The expired breath and urine of nine residents of the Love Canal

TABLE 4-14

Chlorinated Benzenes in the Blood of Nine Residents of Love Canal in Niagara Falls, New York*

Compound	No. of Positive Results	Blood Concentration (ng/mℓ)
Monochlorobenzene	8	0.05-17.0
Di-isomers	9	0.15-68
Tetra-isomers	1	2.6

*Source: Barkley et al., 1980

area contained measurable levels of the chlorobenzenes as shown in Table 4-15 (Barkley et al., 1980).

The concentrations of chlorinated benzenes reported for human tissue (see Table 4-13) and blood, breath and urine (see Tables 4-14 and 4-15) indicate that humans absorb chlorinated benzenes. The bioaccumulation of the chlorinated benzenes is offset by metabolism and elimination from the body.

4.4. RELATIVE SOURCE CONTRIBUTIONS TO TOTAL EXPOSURE

The monitoring studies discussed in the preceding section indicate that chlorinated benzenes are present in the environment and that human exposure to one or more of these substances is likely to result from the inhalation of air or the ingestion of water or food. The intent of this section is to estimate the relative degree that these three media -- air, water and food -- contribute to a person's overall exposure. There are several limitations to this approach. First, no comprehensive study of human exposure to the chlorobenzenes has been conducted; the available monitoring data indicate the presence of the substances under the conditions of a given study and do not establish universal levels of exposure. Consequently, the studies that are used in this section were selected on the basis of being the most likely to represent general population exposure. Data on instances of gross contamination; i.e., local pollution from landfill or in an occupational setting, were not used. Second, no single study has analyzed any one medium for all of the chlorobenzenes. Hence, only data from a single study were used in the calculations for one type of exposure; aggregate or combined data were not used. Third, all monitoring studies are limited either on terms of sampling duration or the number of locations sampled; studies with the widest geographical sampling locations and longest duration of sampling

TABLE 4-15

Chlorinated Benzenes in the Breath and Urine of Nine Residents of Love Canal in Niagara Falls, New York*

Compound	No. of Positive Results		Concentration Range	
	Breath	Urine	Breath (ng/m³)	Urine (ng/ℓ)
Monochlorobenzene	1	6	T	20-120
Di-isomers	7	7	T-5000	40-39,000
Tri-isomers	2	0	T-90	ND
Tetra-isomers	2	0	30-180	ND
Pentachlorobenzene	1	0	70	ND

*Source: Barkley et al., 1980

T = Trace; ND = not detected

were favored for use in the calculations. Finally, quantitative data on the absorption of the various chlorobenzenes by humans through the lungs, skin or gastrointestinal tract is not available. For this reason, the data in this section are estimates of yearly average ambient exposure levels (i.e., the amount potentially inhaled or ingested) and are not physiological exposure levels.

4.4.1. Air. The monitoring data used for the estimation of inhalation exposure (Table 4-16) are taken from the overall mean concentration values given in Table 4-7. In addition, this table presents estimates of the total yearly exposure of an adult man, adult woman, child and infant using standard respiratory volumes of 8.4×10^6, 7.7×10^6, 5.5×10^6 and 1.4×10^6 ℓ/year, respectively (ICRP, 1975). The inhalation exposure estimate will be different for rural/remote, urban/suburban and source areas.

4.4.2. Water. The estimation of exposure of chlorobenzenes from drinking water requires that the mean or median concentrations of these compounds in finished water originating from a large number of both U.S. surface and groundwater be known. As discussed in Section 4.3.2., only a limited number of monitoring data for the levels of chlorobenzenes in finished water samples are available. Therefore, an assessment of the exposure of chlorobenzenes through the ingestion of drinking water cannot be made at the present time. However, the maximum concentrations of the chlorobenzenes found in U.S. drinking water are the following: monochlorobenzene, 5.6 μg/ℓ; 1,3-dichlorobenzene, <3 μg/ℓ; and trichlorobenzene (isomer unspecified), 1.0 μg/ℓ (NAS, 1977). If the maximum fluid intake by an individual is assumed to be 711.8 ℓ/year (ICRP, 1975), the maximum exposure of an individual chlorobenzene isomer through ingestion of finished water can be estimated to be <4 mg/year.

TABLE 4-16

Estimated Yearly Exposure to Several Chlorinated Benzenes Via Inhalation

Chemical	Mean Ambient Concentration (ng/m³)*	Exposure (mg/yr) Adult Man	Exposure (mg/yr) Adult Woman	Exposure (mg/yr) Child (10 yr)	Exposure (mg/yr) Infant (1 yr)
Monochlorobenzenes	3087	25.9	23.8	17.0	4.3
1,2-Dichlorobenzene	1142	9.6	8.8	6.3	1.6
1,3-Dichlorobenzene	571	4.8	4.4	3.1	0.8
1,4-Dichlorobenzene	1563	13.1	12.0	8.6	2.2
Trichlorobenzenes	136	1.1	1.0	0.7	0.2
Tetrachlorobenzenes	3502	29.4	27.0	19.3	4.9

*Mean levels obtained from Table 4-8

4.4.3. Food. Hexachlorobenzene is the only chlorinated benzene whose presence in food has been systematically investigated. Based on data from the Total Diet Program, the FDA estimated the average daily intake of hexachlorobenzene for fiscal year 1974 to be 0.0725 μg/day (IARC, 1979). This would result in a yearly exposure of 0.03 mg hexachlorobenzene from food sources.

4.5. SUMMARY

Annual production of chlorinated benzenes in 1983 is on the order of 450 million pounds, the majority of which is accounted for by monochlorobenzene and dichlorobenzenes. Production of the tri- and tetrachlorobenzenes and pentachlorobenzene is on the order of millions of pounds/year. Hexachlorobenzene is not currently produced as a commercial product in the United States (IARC, 1979), although it is a constituent of several imported products and is a byproduct or waste material in the production of many chemicals (Mumma and Lawless, 1975). These compounds are used in a number of organic chemical syntheses, including the synthesis of other chlorobenzenes, and have applications as solvents, electrical equipment insulators, pesticides, herbicides and fungicides. Emissions of chlorobenzenes are most likely to occur during their manufacture or use as intermediates and from the disposal of waste products from manufacturing operations. Hexachlorobenzene, for example, which is imported but not produced commercially in the United States, occurs as a byproduct in the synthesis of nine other chlorocarbons; 2-5 million pounds may be generated each year.

Chlorinated benzenes have been identified in air, food and soil, and in surface, ground and drinking water. The highest concentrations have been found near manufacturing and waste disposal sites, although no study has attempted to characterize the contribution of any one source to the total environmental contamination by chlorobenzenes. Ambient air and maximum water levels are in the μg/m³ and mg/ℓ range, respectively, although monitoring studies for finished water have been limited. The most frequently detected compounds in air and water were monochlorobenzene and the di- and trichlorobenzenes. Penta- and hexachlorobenzene are more frequently found in food and soil, although their detection may reflect more of the concern over their use as pesticides and fungicides, or their presence as contaminants in pesticides or fungicides, rather than the absence of the other chlorobenzenes.

No comprehensive study of human exposure to the chlorobenzenes has been conducted, although their ubiquity in the environment and the detection of measurable residues in human tissue (see Section 5.3., Bioaccumulation/Bioconcentration) indicate that human exposure and absorption occur. The contribution of the chlorobenzenes from all the three media (air, water and food) to a person's total exposure cannot be made with the limited environmental monitoring data. The available data, however, indicate that human inhalation exposure to chlorobenzenes may be higher than ingestion exposure either through drinking water or through foods.

5. ENVIRONMENTAL TRANSPORT AND FATE

The following sections consider the transport and fate of the chlorinated benzenes through the three environmental media (air, water and soil) and their potential to accumulate or concentrate in plant, animal, and ultimately, human tissues. Transport between the various environmental media is governed by the physical and chemical characteristics of the compounds and their interaction with components of the environment. Evaporation rates and solubilities influence transport from water and soil into air. Leaching rates, adsorption, rainfall, soil type and desorption affect the movement of chlorobenzenes from soil and sediment into water and groundwater, as well as from water into sediment and soil. The fate of chlorobenzenes in the environment depends on degradative processes, either abiotic degradation by chemical reactions or photolysis, or biotic degradation by microbes, and on the rate at which these compounds are stored or accumulated by plants, animals and humans.

5.1. TRANSPORT

5.1.1. Air. The transport and distribution of the chlorobenzenes in the atmosphere has not been investigated. One study has suggested that distribution of one of the chlorobenzenes in air may be global. Atlas and Giam (1981) reported detecting hexachlorobenzene at a mean level of 0.10 ng/m^3 in air samples taken at a remote North Pacific Ocean atoll where the only source could be air transport. These data led the authors to suggest that hexachlorobenzene is well mixed in the atmosphere and has wide distribution in the Northern Hemisphere. A study of environmental contamination by hexachlorobenzene from industrial plants (Li et al., 1976) provided some data that indicated such emissions can be spread by wind from point sources. The authors reported that the emissions, which were in both vapor and particulate form, were detected at levels from 0.1-29.0 μg/m³ near the production facilities and decreased to 0.10-0.50 as much 200 feet or more downwind. The tendency of the hexachlorobenzene to remain in the atmosphere was not studied.

Entry into the atmosphere from other media is determined mainly by a substance's molecular weight, water solubility and vapor pressure. Chlorobenzenes have vapor pressures ranging from 0.05-11.8 mmHg at 20°C (see Section 3.3.). In general, these vapor pressures decrease with the increase in the number of chlorine substituents. Chlorobenzenes are likely to enter the atmosphere as a result of evaporation from soil and water and these types of studies are discussed in the following sections.

5.1.2. **Water.** Chlorobenzenes have low solubility in water, with the solubility decreasing as the number of chlorine substituents increases, although some variation is evident among the isomers (Hawley, 1977; Sax, 1979; Weast, 1979) (see Section 3.3.). Once dissolved in water, despite their relatively low vapor pressures and high molecular weights, the chlorinated benzenes tend to evaporate quickly (Mackay and Wolkoff, 1973). Two laboratory studies indicate that evaporation of some of the chlorobenzenes from an aqueous solution will be as rapid as a few minutes to a few days.

Garrison and Hill (1972) found that >99% of mono-, 1,2- and 1,4-di- and 1,2,4-trichlorobenzene had evaporated within 4 hours from aerated distilled water solutions and within 72 hours from nonaerated solutions. Mono-, 1,2-di- and 1,4-dichlorobenzene volatilized completely in <1 day from aerated solutions containing mixed cultures of aerobic microorganisms. 1,2,4-Trichlorobenzene also evaporated, but less rapidly, with 2% of the initial concentration remaining after 80 hours. Lu and Metcalf (1975) provided evidence of monochlorobenzene's volatility from water through their study of this chemical's biodegradability in a model ecosystem. They noted that after 48 hours, 96% of the radioactively labeled compound added to the model system was found in the traps that sampled the system's atmosphere.

A 1-year field study of the transport of 1,4-dichlorobenzene in Lake Zurich, Switzerland, also indicated an important role for evaporation in the removal of chlorobenzenes from water (Schwarzenbach et al., 1979). The authors found that the main input of 1,4-dichlorobenzene into the lake was from wastewater treatment plants and that the half-life of the chemical was ~100 days. From a comparison of the seasonal variation in evaporation rates, they concluded that transport into the atmosphere is the predominant influence on the loss of 1,4-dichlorobenzene from the lake. Their data indicated that of the 90 kg/year entering the lake, 60 kg was lost to the air, 2 kg entered lake sediments and 28 kg was in the lake's outflow.

In addition to laboratory and field investigations, theoretical studies of the transport of chlorobenzenes in aquatic systems may be useful in predicting the distribution of these compounds and their removal from water by evaporation and sedimentation. Using Henry's Law Constant and various assumptions of water depth, air speed, etc., the half-life of evaporation from water can be calculated. For chlorobenzene, 1,2-dichlorobenzene and 1,2,4-trichlorobenzene, these values are 4.6 minutes, 8.1 minutes and 0.75 hours, respectively. Falco et al. (1982) developed a mathematical model for assessing the transport and degradation of materials released from landfills and waste storage lagoons. The parameters incorporated into the model included coefficients for the following: (1) octanol/water partition, (2) hydrolysis rate, (3) photolysis rate, (4) bacterial degradation rate, (5) oxidation rate, (6) overall degradation rate and (7) volatilization rate. The predictions made using the model are therefore limited by the available data base and rate coefficients. In their modeling, Falco et al. (1982) used the model to predict the transport and persistence of chlorobenzenes for the following types of surface waters: (1) a river capable of transporting a chemical 50-100 miles in 5 days, (2) a pond with an average retention time of 100 days and (3) a lake or reservoir with a retention time of 1 year. For comparative purposes, a summary of the authors' results for the lake or reservoir is presented in Table 5-1.

5.1.3. Soil. Chlorobenzenes have an intermediate to high potential for adsorption onto soils, which tends to increase with increasing number of chlorine substituents. Once adsorbed, their movement within the soil is dependent on the soil type and the nature of the solvent or leachate. In the absence of a solvent, transport into adjacent soil and the atmosphere is likely to result from vapor phase diffusion.

Wilson et al. (1981) studied the transport, over a 21-day period, of a mixture of monochlorobenzene, 1,4-dichlorobenzene, 1,2,4-trichlorobenzene and 10 other organic chemicals through a column of sandy soil having a low organic matter content. Using water as a solvent, these investigators noted that for the chlorobenzenes the retardation factors (velocity of the solvent/velocity of a compound) increased with the chlorine number regardless of the initial concentration of the compounds. These authors also reported that up to 50% of the applied monochlorobenzene evaporated and ~50% of the amount of all three chlorobenzenes was degraded or unaccounted for (Table 5-2). These results indicated that chlorinated benzenes are likely to leach into groundwater and this mobility in groundwater was confirmed in a field study by Roberts et al. (1980).

Studies on the transport of hexachlorobenzene indicate a high potential for soil adsorption and for volatilization from porous soils. Ausmus et al.

TABLE 5-1

Predicted Transport and Fate of Chlorinated Benzenes Released from Landfills and Lagoons[a]

	Percentage of Total Amount of Chlorobenzene Entering Lake				
Property	Mono-	1,2- and 1,4-Di-	Tri-[b]	Tetra-[b]	Penta-
Movement from point of entry to outlet	5-9	<1	5-6	6-8	6-41
Potential for degradation or elimination	83-94	76-97	NG	NG	NG
Amount absorbed onto suspended sediments	<1-8	2-24	13-65	4-37	62-95
Amount taken up by fish	0	0	<1	<1	<1
Estimated volatilization to atmosphere	74-88	1-3	31-81	55-89	0

[a] Source: Falco et al., 1982

[b] Isomer not specified

NG = Not given

TABLE 5-2

Transport of Chlorinated Benzenes in Sandy Soil*

Chemical	Percentage of Total Chlorobenzene Applied		
	Volatilized	Degraded or Not Accounted For	Column Effluent
Monochlorobenzene	27-54	20-40	26-33
1,4-Dichlorobenzene	ND	51-63	37-49
1,2,4-Trichlorobenzene	ND	54-61	39-46

*Source: Wilson et al., 1981

ND = Not determined

(1979) applied C^{14}-labeled hexachlorobenzene to soil cores taken from a pine forest and monitored its evaporation and leaching by water over 21 days. Of the amount applied, <1% was lost by volatilization or in the leachate, and none was degraded as indicated by the absence of labeled CO_2. Farmer et al. (1980a) examined the vapor phase diffusion of hexachlorobenzene through a high clay, low organic material soil (39 and 1%, respectively) and reported diffusion to be increased by the soil porosity and decreased by the soil's water content. The same authors (Farmer et al., 1980b) also found that highly compacted wet soil covers were most effective in reducing hexachlorobenzene volatilization after dumping into a land-fill. A water cover in a temporary storage lagoon was also effective. Each 10°C rise in soil temperature increased volatilization fluxes 3.5-fold. Griffin and Chou (1981) investigated the adsorption and mobility of polychlorinated and polybrominated biphenyls and hexachlorobenzene in seven different soil types with increasing amounts of organic carbon. The adsorption of hexachlorobenzene increased with increasing amounts of organic carbon. Further, they noted that hexachlorobenzene was immobile and was not leached from the three soils that were tested with water and a leachate from a landfill.

K_{oc} values for chlorinated benzenes are: chlorobenzene (537), 1,2-dichlorobenzene (977), 1,4-dichlorobenzene (1259), 1,2,3-trichlorobenzene (2630), 1,2,4-trichlorobenzene (2042) and hexachlorobenzene (38,000) (Calamari et al., 1983).

5.2. FATE

5.2.1. Air. The degradation of chlorobenzenes in air has been studied in a fair amount of detail. In theory, chlorobenzenes dispersed in air may be degraded by chemical- or sunlight-catalyzed reactions or may be adsorbed onto particles that settle or are removed from the atmosphere by rain. A measure of the effectiveness of these factors is the atmospheric residence time. One study has made estimates of residence times for various chlorobenzenes. Singh et al. (1981) conducted field studies in California and Arizona and analyzed air samples over 2-week periods for 33 organic chemicals including monochlorobenzene, the dichlorobenzenes and an unspecified isomer of trichlorobenzene. The estimated residence times of these chemicals and daily percentage of each lost from the atmosphere are presented in Table 5-3.

5.2.2. **Water**. The fate of chlorobenzenes in aquatic systems has not been completely characterized, although initial studies indicate that degradation of chlorobenzenes is possible by microbial communities in wastewater treatment plants and in natural bodies of water. Other investigations have indicated that chlorobenzenes have a high potential for bioaccumulation and bioconcentration by aquatic species (Section 5.3.). Removal of chlorobenzenes by adsorption onto suspended material that in turn settles and is incorporated into sediments has not been demonstrated.

Lee and Ryan (1979) examined the degradation of various chlorinated compounds by microbes in samples of water and sediments taken from a river in Georgia. They observed that the degradation rates fit first-order expressions, although the degradation of the chlorinated compounds in water was slow. In the sediment samples, monochlorobenzene was found to have a half-life of 75 days, which was longer than the chlorinated phenols, but more rapid than the degradation of hexachlorophene and DDE. In contrast, hexachlorobenzene showed no degradation by water or sediment microbes. Davis et al. (1981) conducted a similar experiment using samples of microbial populations from industrial and municipal wastewater treatment plants and 1,2-dichlorobenzene along with other compounds. The dichlorobenzene at a concentration of 50 ng/ℓ was degraded by both systems within 7 days.

TABLE 5-3

Estimated Atmospheric Residence Time and Daily Loss Rates for Several Chlorinated Benzenes[a]

Chemical	Residence Times (days)[b]	Daily Loss Rate (percent)[c]
Monochlorobenzene	13	7.4
1,2-Dichlorobenzene	38.6	2.6
1,3-Dichlorobenzene	38.6	2.6
1,4-Dichlorobenzene	38.6	2.6
Trichlorobenzene[d]	116.0	0.9

[a] Source: Singh et al., 1981

[b] Calculated assuming an average daily (24-hour) abundance of OH radicals of 10^6 molecules/cm^3

[c] For 12 hours of sunlight

[d] Isomer unspecified

This rate, which was more rapid than the rate for phenol but slower than that measured for benzene, was described as "comparatively rapid."

Using a model aquatic ecosystem, Lu and Metcalf (1975) investigated the biodegradation and bioaccumulation of various chemicals including monochlorobenzene and hexachlorobenzene. Both compounds were found to have high "ecological magnification" indices and accumulated in both aquatic plants and animals. Both chemicals had low biodegradability indices and were, in general, metabolized to monochloro- and pentachlorophenol, respectively.

A number of other investigators have studied the biodegradability of chlorinated benzenes and these results are summarized in Table 5-4. In general, these results suggest that the biodegradability decreases as the number of chlorine substituents increases. In addition to these laboratory studies, Zoeteman et al. (1980) indicated that chlorobenzene, o-dichlorobenzene, p-dichlorobenzene, 1,2,4-trichlorobenzene and hexachlorobenzene degrade in river water with half-lives of 0.30, 3-2, 1.1-25, 1.8-28 and 0.5 days, respectively, as indicated by monitoring at various stations along the Rhine River. These half-lives are likely to be very inaccurate since only a limited number of samples were taken.

Roberts et al. (1980) studied the transport and degradation of monochlorobenzene, o-, m-, p-chlorobenzenes and 1,2,4-trichlorobenzene in groundwater after injection by analyzing monitoring wells at different distances from the injection well. No degradation was noted.

5.2.3. Soil. Studies on the fate of dichlorobenzenes, trichlorobenzenes, pentachlorobenzene and hexachlorobenzene in soil have indicated that the chlorobenzenes are usually resistant to microbial degradation (however, compare Ballschmiter and Scholz, 1980) and that chlorophenols are likely degradation products. Beck and Hansen (1974) studied the biodegradation of

TABLE 5-4

Aqueous Biodegradability Studies of Chlorinated Benzenes

Method	Results (% Degradation)					Reference
	MCB	o-DCB	p-DCB	1,2,4-TCB	HCB	
Warburg	3.9 BODT		Trace of degradation	3.4 BODT	No degradation	Malaney and McKinney, 1966
Mineral salts shake flask	100*	18-66*	0-61*	0-70*	0-56*	Tabak et al., 1981
MITI BOD Test	Resistant to degradation	Resistant to degradation	Resistant to degradation			Kawasaki, 1980
Warburg (phenol acclimated cultures)	16.1 BODT	2.4 BODT				Chambers et al., 1963
BOD 5-day	1.5 BODT					Heukelekiah and Rand, 1965
Natural water	Degradation fast in fresh water, slower in estuarine and marine water			slow degradation		Pfaender and Bartholomew, 1982
Warburg (sewage)				0-54		Gaffney, 1976

*Percent degradation after acclimation (subculture every 7 days)

Quintozene, a fungicide, and two of its impurities, penta- and hexachlorobenzene. Soil samples treated under laboratory conditions with penta- and hexachlorobenzene at rates equal to 10 mg/ha were monitored over a period of 600 days. From the slopes of the degradation curves, the authors estimated the half-lives of penta- and hexachlorobenzene to be 194-345 and 969-2089 days, respectively. Beall (1976) applied hexachlorobenzene at an amount equivalent to 750 g/ha to sections of turf in a greenhouse. Within 2 weeks, 55% of the hexachlorobenzene had disappeared from the top 2 cm of soil, most likely a result of evaporation. Very little of the chemical disappeared from the 2-4 cm-deep soil layer over the next 19 months. Isensee et al. (1976) also found hexachlorobenzene to be highly persistent in soil. Hexachlorobenzene was applied to samples of sterile and nonsterile soil to create levels of 0.1, 1, 10 and 100 ppm. After storage of the samples under aerobic (sterile and nonsterile) and anaerobic (nonsterile) conditions for 1 year, analysis indicated that none of the hexachlorobenzene had degraded in any sample.

Studies with the di- and trichlorobenzenes have indicated that these compounds are also persistent, but not to the degree reported for hexachlorobenzene. Ballschmiter and Scholz (1980) investigated the metabolism of 1,2-, 1,3- and 1,4-dichlorobenzene by a soil microbe of the *Pseudomonas* genera. In culture, the soil microbe was capable of degrading the compounds to dichlorophenols and dichloropyrocatechols. Similar cultures of *Pseudomonas* also metabolized the tri- and tetrachlorobenzenes to their respective chlorophenols. In an experiment that more closely duplicated conditions in nature, Marinucci and Bartha (1979) treated fresh field soil with radiolabeled 1,2,3- and 1,2,4-trichlorobenzene. They found very slow rates of degradation for these compounds, 0.35 and 1.00 nmol/day/20 g of soil, respectively. These authors also noted that the amount of organic material in the soil had no effect on the rate, but it did appear to reduce evaporation of the chlorobenzenes. The primary degradation products were chlorophenols. Haider et al. (1974), using ^{14}C-labeled compounds in soil, found 18.3, 1.1 and 1.1% CO_2 after 1 week of incubation of monochlorobenzene, o-dichlorobenzene and p-chlorobenzene, respectively.

5.3. BIOCONCENTRATION, BIOACCUMULATION, AND BIOMAGNIFICATION

The occurrence of toxic substances in the environment raises the issues of whether humans may be exposed to them via air, water or food and, if so, what are the physiological exposures. The transport and fate of the chlorobenzenes (see Sections 5.1. and 5.2.) are primary determinants of human exposure to the environmental sources of these compounds, but the more crucial physiological exposure levels are determined by the ease with which a compound crosses biological membranes. Bioaccumulation, alternately expressed as biological persistence, is the net result of the absorption and the elimination rate of a compound and, therefore, determines the level and duration of human physiological exposure.

The terminology used in this section will follow the suggestion of Macek et al. (1979): bioconcentration implies that tissue residues result only from exposure to the ambient environment (i.e., air for terrestrial or water for aquatic species); bioaccumulation considers all exposures (air, water and food) of an individual organism as the source of tissue residues; and biomagnification defines the increase in tissue residues observed at successively higher trophic levels of a food web.

Tissue concentrations of the various chlorinated benzenes in laboratory and field populations are discussed in Chapter 6, Ecological Effects. It is sufficient to state here that the chlorinated benzene isomers do reach measurable tissue levels in exposed organisms. The factors controlling their accumulation, however, are pertinent for discussion in this section.

Studies of the accumulation of xenobiotics from environmental sources into living cells and tissues have been conducted mainly with aquatic species and food chains. Under the general experimental design, the organisms are exposed to sublethal concentrations of the test material under static or flowing water conditions. After exposure, the concentration of the test material in the organism is quantified and a bioconcentration factor (BCF) is calculated as the ratio of the concentration in tissue (or the whole organism) to the concentration in the water or food; air concentration is substituted into the denominator for calculating the BCF for terrestrial organisms (Macek et al., 1979; Veith et al., 1980).

From such studies, it appears that concentration is determined by water solubility, the octanol/water partition coefficient (Lu and Metcalf, 1975) or the number of chlorine atoms on the molecule (Barrows et al., 1980). All three parameters correlate well with the BCF (Kenaga and Goring, 1980; Metcalf, 1977; Lu and Metcalf, 1975). Table 5-5 shows the direct relationships between increasing chlorination, increasing lipid solubility as indicated by the octanol/water partition coefficient and the increase in the BCF for chlorinated benzenes in fish. For example for salmon (Oliver and Nimmi, 1983) all the chlorobenzenes except hexachlorobenzene obeyed:

$$\log \text{BCF} = -0.632 + (1.022 \pm 0.057) \log K \text{ at the high exposure end,}$$

and

$$\log \text{BCF} = -0.869 + (0.997 \pm 0.056) \log K \text{ at the low exposure end.}$$

Accordingly, the octanol/water partition coefficient is a good first approximation of the BCF in aquatic organisms ($r = 0.948$, $n = 8$ in flowing water) (Kenaga and Goring, 1980).

TABLE 5-5

Octanol/Water Partition Coefficients, Bioconcentration Factors and Biological Half-lives for Chlorinated Benzenes in Fish

Compound	Octanol/Water Partition Coefficient[a]	Species	BCF[b]	Biological Half-life[c] (days)	Reference
Monochlorobenzene	690	NS	12	NR	Kenaga and Goring, 1980[d]
	NR	fathead minnow	450	NR	Veith et al., 1979
	NR	rainbow trout	46	NR	Branson, 1978
1,2-Dichlorobenzene	2,511	bluegill	89	<1	Veith et al., 1980
	2,510	rainbow trout	270-560	NR	Oliver and Niimi, 1983
1,3-Dichlorobenzene	2,754	bluegill	66	<1	Veith et al., 1980
	2,750	rainbow trout	420-740	NR	Oliver and Niimi, 1983
1,4-Dichlorobenzene	2,400	bluegill	15	<7	U.S. EPA, 1980
	2,344	bluegill	60	<1	Veith et al., 1980
	2,400	rainbow trout	214	NR	Neely et al., 1974
	2,340	rainbow trout	370-720	NR	Oliver and Niimi, 1983
	NR	rainbow trout	32-107	<1	Galassi et al., 1982
	NR	rainbow trout	80	NR	Calamari et al., 1982
	2,400	trout	231	NR	U.S. EPA, 1980
	3,388	guppy	100	0.7	Konemann and Van Leeuwen, 1980
1,2,4-Trichlorobenzene	NR	fathead minnow	1700	<7	Kosian et al., 1981
	17,000	fathead minnow	2100	NR	Veith et al., 1979
	17,000	green sunfish	2300	NR	Veith et al., 1979
	NR	bluegill	182	>1<3	Barrows et al., 1980
	17,000	rainbow trout	890	NR	Veith et al., 1979
	10,500	rainbow trout	1300-3200	NR	Oliver and Niimi, 1983
	15,000	NS	491	NR	Kenaga and Goring, 1980
1,3,5-Trichlorobenzene	14,100	rainbow trout	1800-4100	NR	Oliver and Niimi, 1983
	15,850	guppy	760	1.7	Konemann and Van Leeuwen, 1980
1,2,3-Trichlorobenzene	12,900	rainbow trout	1200-2600	NR	Oliver and Niimi, 1983
	15,850	guppy	700	1.5	Konemann and Van Leeuwen, 1980
1,2,3,5-Tetrachlorobenzene	28,800	bluegill	1800	>2<4	Veith et al., 1980
	87,100	guppy	3900	2.5	Konemann and Van Leeuwen, 1980

TABLE 5-5 (cont.)

Compound	Octanol/Water Partition Coefficient[a]	Species	BCF[b]	Biological Half-life[c] (days)	Reference
1,2,4,5-Tetrachlorobenzene	33,100	rainbow trout	5300-13,000	NR	Oliver and Niimi, 1983
	47,000[e]	NS	4500	NR	Kenaga and Goring, 1980
	NR	carp	4000-4900	NR	Kitano, 1978
1,2,3,4-Tetrachlorobenzene	28,800[e]	rainbow trout	5200-12,000	NR	Oliver and Niimi, 1983
	NR	carp	3800-4500	NR	Kitano, 1978
Pentachlorobenzene	87,096	bluegill	3400	>7	Veith et al., 1980
	87,100[f]	rainbow trout	13,000-20,000		Oliver and Niimi, 1983
	154,000	NS	~5000		Kenaga and Goring, 1980
	490,000	guppy	14,000	3.8	Konemann and Van Leeuwen, 1980
Hexachlorobenzene	NR	fathead minnow	35,000	>7<21	Kosian et al., 1981
	170,000	fathead minnow	16,200-18,500	NR	Veith et al., 1979
	170,000	green sunfish	21,900	NR	Veith et al., 1979
	170,000	rainbow trout	5500	NR	Veith et al., 1979
	169,824	rainbow trout	7762	NR	Neely et al., 1974
	316,000[g]	rainbow trout	12,000-20,000	NR	Oliver and Niimi, 1983
	168,000	NS	8600	NR	Kenaga and Goring, 1980
	NR	largemouth bass	18,214-44,437	>4<9	Laseter et al., 1976
	NR	sheepshead minnow	20,000	NR	Parrish et al., 1978

[a] Determined experimentally or by calculation from relative chromatographic retention time

[b] Tissue concentration/water concentration (in flowing water)

[c] Depuration time for tissue concentration to decrease by one-half

[d] Kenaga and Goring (1980) reported these data from various authors; therefore, each entry in a row may be from a different study.

[e] Konemann et al. (1979)

[f] Banerjee et al. (1980)

[g] Chiou et al. (1982)

NR = Not reported; NS = Not specified

Bioaccumulation in aquatic species is a function of the total environmental exposure of the organism including both water and the food consumed. Macek et al. (1979) showed, however, that uptake of 1,2,4-trichlorobenzene from the ambient water (bioconcentration) accounted for 93% of the total body burden, while diet accounted for 6-7% of the ^{14}C-1,2,4-trichlorobenzene measured in bluegills, Lepomis macrochirus, after 28 days of exposure. Similar conclusions were reached by Laseter et al. (1976) using bass and bluegills exposed to hexachlorobenzene.

Although the chlorinated benzenes do bioaccumulate and establish tissue concentrations in equilibrium with the environment (Kenaga and Goring, 1980; Veith et al., 1980; Lu and Metcalf, 1975), the biological (referring to individual organisms) and ecological (referring to biomagnification) persistence of the substance may be the more important parameter. The longer biological half-life of persistent compounds is most likely a result of their relative tissue-binding kinetics and the rate of their biotransformation. Ware and West (1977) concluded that halogenation of a compound increased its resistance to biotransformation. This, together with the high affinity for adipose tissue, suggests that the chlorobenzenes are persistent compounds; this is shown for fish in Table 5-5.

The extent of halogenation also affects the rate of depuration. Guppies, Poecilia reticulata, were exposed to a standardized mixture of six chlorobenzenes for 19 days and then allowed to depurate for 9 weeks (Konemann and Van Leeuwan, 1980). The ambient water concentration of each chlorobenzene, the BCF and the slope of the elimination curve are shown in Table 5-6. While the chlorobenzenes as a group are persistent, halogenation influences their rate of elimination.

TABLE 5-6

Bioconcentration Factor and Slope of the Elimination Curve for Guppies (Poecilia reticulata) Exposed to Six Chlorinated Benzenes[a]

Compound	Average Concentration Measured in Water (ng/mℓ)	BCF[b]	Slope of Elimination Curve (day^{-1})[c]
1,4-Dichlorobenzene	116	1.8×10^3	1.00
1,2,3-Trichlorobenzene	48	1.3×10^4	0.45
1,3,5-Trichlorobenzene	43	1.4×10^4	0.40
1,2,3,5-Tetrachlorobenzene	12	7.2×10^4	0.28
Pentachlorobenzene	1.2	2.6×10^5	0.18
Hexachlorobenzene	0.3	2.9×10^5	0.062

[a] Source: Konemann and Van Leeuwan, 1980

[b] Calculated on the basis of fat weight (average fat weight = 5.4%)

[c] Only 1,4-di- and hexachlorobenzene had single-phase elimination curves; the second-phase slopes for the other compounds are excluded for simplicity.

The significance of biological persistence lies in the increased time of physiological exposure for an individual organism and the greater probability for human exposure via environmental media.

No studies were available on the bioaccumulation of the chlorobenzenes in terrestrial food webs. There are, however, no immediately apparent reasons why the relationships between bioaccumulation and the physicochemical parameters demonstrated for aquatic systems are not applicable to the terrestrial environment. Generally, for air-breathing terrestrial species such as humans, the atmospheric concentration of a compound is the primary determinant of bioaccumulation because the frequency of air intake is much greater compared to the ingestion of food or water. This was apparent from the analysis of ambient air and household tapwater samples taken from nine homes in the Love Canal area of Niagara Falls, New York (Barkley et al., 1980). From these data, the expected total daily intake of dichlorobenzenes by a 70 kg adult male for example, was nearly 300-fold greater from air (0.119 mg/day) than from tapwater (3.36×10^{-4} mg/day). This topic is discussed more extensively in Sections 4.3. and 4.4.

Although the chlorobenzenes are volatile compounds and inhalation is the expected primary route of human exposure, potentially high intake by other routes cannot be ignored. Therefore, bioaccumulation and internal exposure are multifactorial parameters dependent upon the chlorinated benzene concentration in each of three environmental compartments and upon the relative rate of absorption and elimination for each compound.

5.4. SUMMARY

The chlorinated benzenes are a group of volatile compounds readily evaporated to the atmosphere from soil and water. Point source releases of the chlorinated benzenes are readily carried by prevailing winds and may be the primary source of measurable hexachlorobenzene in industrially remote areas, although there also may be concentration gradients around these point sources. The high vapor pressure and low water solubility of these compounds promotes their release to the atmosphere from open water systems or their association with organic material that may either be incorporated into sediments or flow out of the system. Soils, depending on their type, readily evaporate chlorobenzenes from pore spaces to the atmosphere, or, depending on the relative affinity of the compound, release it as leachate.

Little information is available on the fate of the chlorinated benzenes in air, but one study concluded that the atmospheric residence time increased with an increase in chlorine substituents. Laboratory studies with smog chambers suggests photocatalysis may produce nitrobenzene, and nitrophenol or polychlorinated biphenyls (Dilling et al., 1976; Kanno and Nojima, 1979; Uyeta et al., 1976). The fate of the chlorobenzenes in water and soil are similar, but the rates differ for each process (i.e., biodegradation, loss to the atmosphere, accumulation in the biota, physical removal by outflow or leaching, or sequestration of the unaltered compound).

The chlorobenzenes are lipophilic compounds that bioaccumulate in animal and human tissues from ambient air, water and food. The BCF (tissue concentration/media concentration) is an indicator of bioaccumulation and is determined by physiochemical parameters such as the water solubility, the octanol/water partition coefficient and the number of substituent chlorine atoms (Kenaga and Goring, 1980). Physiological exposure levels are determined by absorption, distribution, metabolism, elimination, and storage in adipose tissue; thus, biologically persistent compounds, such as the chlorobenzenes, may produce prolonged physiological exposures.

6. ECOLOGICAL EFFECTS

As mentioned briefly in the previous chapters, chlorinated benzenes occur in both the aquatic and terrestrial environments. The concentrations of these compounds in some areas suggest that wildlife may be exposed to higher levels of chlorinated benzenes than those encountered by humans. Although aquatic and terrestrial organisms are exposed, no data are available on the toxic effects of chlorobenzenes at environmental concentrations in natural populations. Laboratory testing has shown that chlorobenzenes have toxic effects on aquatic and terrestrial species and bioaccumulate in exposed organisms.

6.1. EFFECTS ON THE AQUATIC ENVIRONMENT

Data on the effects of chlorinated benzenes on freshwater or marine organisms in their natural environment were not available. Chlorobenzenes have been shown to be acutely toxic to aquatic species in laboratory bioassays. The results of such acute toxicity bioassays can be used to determine relative toxicities of the various chlorobenzenes to various species.

6.1.1. Effect on Freshwater and Marine Fish. The acute toxicity of monochlorobenzene has been reported for several species of freshwater and marine fish (Table 6-1). The most sensitive species appears to be the rainbow trout, _Salmo gairdneri_, with 96-hour median lethal concentration (LC_{50}) values ranging between 3-5 mg monochlorobenzene/ℓ (Brosier, 1972; Dow Chemical Company, 1978b; Calamari et al., 1983; Dalich et al., 1982). Bluegill sunfish (_Lepomis macrochirus_), fathead minnows (_Pimephales promelas_) and guppies (_Lebistes reticulatus_) were moderately tolerant with mean 96-hour LC_{50} values ranging from 15.9-24.0, 31.5-33.9 and 45.5 mg/ℓ, respectively (U.S. EPA, 1978; Pickering and Henderson, 1966). The goldfish, _Carassius auratus_, was the species most tolerant of monochlorobenzene with a 96-hour LC_{50} value of 51.62 mg/ℓ (Pickering and Henderson, 1966).

TABLE 6-1

Acute Toxicity Data for Fish Species Exposed to Chlorinated Benzenes

Compound	Species	Duration (hour)	Mean Concentration (mg/ℓ)	Method	Effect	Reference
Monochlorobenzene	rainbow trout (Salmo gairdneri)	96	3.58	constant-flow	LC_{50}	Dow Chemical Co., 1978b
		24	1.8	NR	LC_{50}	Gingerich and Dalich, 1978
		8	5-10	static	LC_{100}	Brosier, 1972
		96	3-5	static	LC_{50}	Brosier, 1972
		96	4.7	constant-flow	LC_{50}	Dalich et al., 1982
		48	4.1^{a}	IRSA	LD_{50}	Calamari et al., 1983
	bluegill sunfish (Lepomis macrochirus)	24	16.9	static	LC_{50}	U.S. EPA, 1978
		48	15.9	static	LC_{50}	U.S. EPA, 1978
		72	15.9	static	LC_{50}	U.S. EPA, 1978
		96	15.9	static	LC_{50}	U.S. EPA, 1978
		96	7.80	static	None	U.S. EPA, 1978
		24	24.00	static	$LC_{50}{}^{b}$	Pickering and Henderson, 1966
		48	24.00	static	$LC_{50}{}^{b}$	Pickering and Henderson, 1966
		96	24.00	static	$LC_{50}{}^{b}$	Pickering and Henderson, 1966
		24	17.0	static	LC_{50}	Buccafusco et al., 1981
		96	16.0	static	LC_{50}	Buccafusco et al., 1981
	fathead minnows (Pimephales promelas)	24	31.53	static	$LC_{50}{}^{b}$	Pickering and Henderson, 1966
		48	31.53	static	$LC_{50}{}^{b}$	Pickering and Henderson, 1966
		96	31.53	static	$LC_{50}{}^{b}$	Pickering and Henderson, 1966
		24	39.19	static	$LC_{50}{}^{c}$	Pickering and Henderson, 1966
		48	34.98	static	$LC_{50}{}^{c}$	Pickering and Henderson, 1966
		96	33.93	static	$LC_{50}{}^{c}$	Pickering and Henderson, 1966

TABLE 6-1 (cont.)

Compound	Species	Duration (hour)	Mean Concentration (mg/ℓ)	Method	Effect	Reference
Monochlorobenzene (cont.)	goldfish (*Carassius auratus*)	24	73.03	static	LC_{50}[b]	Pickering and Henderson, 1966
		48	56.00	static	LC_{50}[b]	Pickering and Henderson, 1966
		96	51.62	static	LC_{50}[b]	Pickering and Henderson, 1966
	guppies (*Lebistes reticulatus*)	24	45.53	static	LC_{50}	Pickering and Henderson, 1966
		48	45.53	static	LC_{50}	Pickering and Henderson, 1966
		96	45.53	static	LC_{50}	Pickering and Henderson, 1966
	sheepshead minnow (*Cyprinodon variegatus*)	24	>19.9	static	LC_{50}	U.S. EPA, 1978; Heitmuller et al., 1981
		48	8.94	static	LC_{50}	
		96	10.50	static	LC_{50}	
		96	6.20	static	None	
	Brachydanio rerio	48	10.5[a]	IRSA	LC_{50}	Calamari et al., 1983
1,2-Dichlorobenzene	rainbow trout (*Salmo gairdneri*)	96	1.67	constant-flow	LC_{50}	Dow Chemical Co., 1978b
		48	2.3[a]	IRSA	LC_{50}	Calamari et al., 1983
	bluegill sunfish (*Lepomis macrochirus*)	24	6.26	static	LC_{50}	U.S. EPA, 1978
		48	6.06	static	LC_{50}	U.S. EPA, 1978
		72	5.59	static	LC_{50}	U.S. EPA, 1978
		96	5.59	static	LC_{50}	U.S. EPA, 1978
		96	<3.20	static	None	U.S. EPA, 1978
		24	6.3	static	LC_{50}	Buccafusco et al., 1981
		96	5.6	static	LC_{50}	Buccafusco et al., 1981
		96[d]	27.0	static	LC_{50}	Dawson et al., 1977
	fathead minnow (*Pimephales promelas*)	96	57.0	static	LC_{50}	Curtis and Ward, 1981
		48	76.3	static	LC_{50}	Curtis et al., 1979
		96	57.0	static	LC_{50}	Curtis et al., 1979

TABLE 6-1 (cont.)

Compound	Species	Duration (hour)	Mean Concentration (mg/ℓ)	Method	Effect	Reference
1,2-Dichlorobenzene (cont.)	tidewater silverside (*Menidia beryllina*)	96^d	7.3	static	LC_{50}	Dawson et al., 1977
	sheepshead minnow (*Cyprinodon variegatus*)	24	9.66-12.9	static	LC_{50}	U.S. EPA, 1978; Heitmuller et al., 1981
		48	9.26	static	LC_{50}	
		72	9.66	static	LC_{50}	
		96	9.66	static	LC_{50}	
		96	7.22	static	None	
	Brachydanio rerio	48	6.8^a	IRSA	LC_{50}	Calamari et al., 1983
1,3-Dichlorobenzene	bluegill sunfish (*Lepomis macrochirus*)	24	21.8	static	LC_{50}	U.S. EPA, 1978
		48	10.7	static	LC_{50}	U.S. EPA, 1978
		72	5.02	static	LC_{50}	U.S. EPA, 1978
		96	5.02	static	LC_{50}	U.S. EPA, 1978
		96	1.70	static	None	U.S. EPA, 1978
		24	22.0	static	LC_{50}	Buccafusco et al., 1981
		96	5.0	static	LC_{50}	Buccafusco et al., 1981
	fathead minnow (*Pimephales promelas*)	96	12.7	static	LC_{50}	Curtis and Ward, 1981
	sheepshead minnow (*Cyprinodon variegatus*)	24	8.46	static	LC_{50}	U.S. EPA, 1978; Heitmuller et al., 1981
		48	8.04	static	LC_{50}	
		72	8.04	static	LC_{50}	
		96	7.77	static	LC_{50}	
		96	4.18	static	None	
1,4-Dichlorobenzene	bluegill sunfish (*Lepomis macrochirus*)	24	4.54	static	LC_{50}	U.S. EPA, 1978
		48	4.37	static	LC_{50}	U.S. EPA, 1978
		72	4.37	static	LC_{50}	U.S. EPA, 1978
		96	4.28	static	LC_{50}	U.S. EPA, 1978
		96	<2.80	static	None	U.S. EPA, 1978
		24	4.5	static	LC_{50}	Buccafusco et al., 1981
		96	4.3	static	LC_{50}	Buccafusco et al., 1981

TABLE 6-1 (cont.)

Compound	Species	Duration (hour)	Mean Concentration (mg/ℓ)	Method	Effect	Reference
1,4-Dichlorobenzene (cont.)	fathead minnow (Pimephales promelas)	96	30.0	static	LC_{50}	Curtis and Ward, 1981
		24	35.4	static	LC_{50}	Curtis et al., 1979
		48	35.4	static	LC_{50}	Curtis et al., 1979
		96	33.7	static	LC_{50}	Curtis et al., 1979
	sheepshead minnow (Cyprinodon variegatus)	24	7.5-10.0	static	LC_{50}	U.S. EPA, 1978; Heitmuller et al., 1981
		48	7.17	static	LC_{50}	
		72	7.40	static	LC_{50}	
		96	7.40	static	LC_{50}	
		96	5.6	static	None	
	rainbow trout (Salmo gairdneri)	48	1.18[a]	IRSA	LC_{50}	Calamari et al., 1983
	Brachydanio rerio	48	4.25[a]	IRSA	LC_{50}	Calamari et al., 1983
1,2,3-Trichlorobenzene	rainbow trout (Salmo gairdneri)	48	0.71[a]	IRSA	LC_{50}	Calamari et al., 1983
	Brachydanio rerio	48	3.1[a]	IRSA	LC_{50}	Calamari et al., 1983
1,2,4-Trichlorobenzene	rainbow trout (Salmo gairdneri)	48	1.95[a]	IRSA	LC_{50}	Calamari et al., 1983
	Brachydanio rerio	48	6.3[a]	IRSA	LC_{50}	Calamari et al., 1983
	bluegill sunfish (Lepomis macrochirus)	24	109.0	static	LC_{50}	U.S. EPA, 1978
		48	13.0	static	LC_{50}	U.S. EPA, 1978
		72	3.59	static	LC_{50}	U.S. EPA, 1978
		96	3.36	static	LC_{50}	U.S. EPA, 1978
		96	<1.70	static	None	U.S. EPA, 1978
		24	109.0	static	LC_{50}	Buccafusco et al., 1981
		96	3.4	static	LC_{50}	Buccafusco et al., 1981
	sheepshead minnow (Cyprinodon variegatus)	24	>46.8	static	LC_{50}	U.S. EPA, 1978; Heitmuller et al., 1981
		48	>46.8	static	LC_{50}	
		72	>46.8	static	LC_{50}	
		96	21.4	static	LC_{50}	
		96	14.6	static	None	

TABLE 6-1 (cont.)

Compound	Species	Duration (hour)	Mean Concentration (mg/ℓ)	Method	Effect	Reference
1,2,3,5-Tetrachlorobenzene	bluegill sunfish (*Lepomis macrochirus*)	24	57.8	static	LC_{50}	U.S. EPA, 1978
		48	11.5	static	LC_{50}	U.S. EPA, 1978
		72	8.34	static	LC_{50}	U.S. EPA, 1978
		96	6.42	static	LC_{50}	U.S. EPA, 1978
		96	<1.70	static	None	U.S. EPA, 1978
		24	59.0	static	LC_{50}	Buccafusco et al., 1981
		96	6.4	static	LC_{50}	Buccafusco et al., 1981
	sheepshead minnow (*Cyprinodon variegatus*)	24	>7.5	static	LC_{50}	U.S. EPA, 1978; Heitmuller et al., 1981
		48	5.59	static	LC_{50}	
		72	4.68	static	LC_{50}	
		96	3.67	static	LC_{50}	
		96	1.0	static	None	
1,2,4,5-Tetrachlorobenzene	bluegill sunfish (*Lepomis macrochirus*)	24	5.69	static	LC_{50}	U.S. EPA, 1978
		48	4.35	static	LC_{50}	U.S. EPA, 1978
		72	1.55	static	LC_{50}	U.S. EPA, 1978
		96	1.55	static	LC_{50}	U.S. EPA, 1978
		96	0.68	static	None	U.S. EPA, 1978
		24	5.7	static	LC_{50}	Buccafusco et al., 1981
		96	1.6	static	LC_{50}	Buccafusco et al., 1981
	sheepshead minnow (*Cyprinodon variegatus*)	24	>1.80	static	LC_{50}	U.S. EPA, 1978; Heitmuller et al., 1981
		48	0.90	static	LC_{50}	
		72	0.84	static	LC_{50}	
		96	0.84	static	LC_{50}	
		96	0.32	static	None	
		96	0.33	flowthrough	LC_{50}	Ward et al., 1981
Pentachlorobenzene	bluegill sunfish (*Lepomis macrochirus*)	24	2.27	static	LC_{50}	U.S. EPA, 1978
		48	0.55	static	LC_{50}	U.S. EPA, 1978
		72	0.30	static	LC_{50}	U.S. EPA, 1978
		96	0.25	static	LC_{50}	U.S. EPA, 1978
		96	<0.088	static	None	U.S. EPA, 1978
		24	2.30	static	LC_{50}	Buccafusco et al., 1981
		96	0.25	static	LC_{50}	Buccafusco et al., 1981

TABLE 6-1 (cont.)

Compound	Species	Duration (hour)	Mean Concentration (mg/ℓ)	Method	Effect	Reference
Pentachlorobenzene (cont.)	sheepshead minnow (*Cyprinodon variegatus*)	24	>32.0	static	LC_{50}	U.S. EPA, 1978; Heitmuller et al., 1981
		48	9.55	static	LC_{50}	
		72	3.2-10.0	static	LC_{50}	
		96	0.83	static	LC_{50}	
		96	0.32	static	None	
Hexachlorobenzene	largemouth bass (*Micropterus salmoides*)	240	0.009-0.01	static	None	Laska et al., 1978
		360	0.022-0.026	static	None	Laska et al., 1978
	sheepshead minnow (*Cyprinodon variegatus*)	96	0.13	constant-flow	None	Parrish et al., 1974
	pinfish (*Lagodon rhomboides*)	96	1.0[e]	constant-flow	None	Parrish et al., 1974
	rainbow trout (*Salmo gairdneri*)	48	<0.03[a]	IRSA	LC_{50}	Calamari et al., 1983
	Brachydanio rerio	48	<0.03[a]	IRSA	LC_{50}	Calamari et al., 1983

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[a] Soft water conditions: pH = 7.4; hardness = 320 mg $CaCO_3$/ℓ; oxygen = ≥70%; temperature = 15°C for trout and 23°C for *Brachydanio*

[b] Soft water conditions: pH = 7.5; alkalinity = 18 mg/ℓ; hardness = 20 mg/ℓ

[c] Hard water conditions: pH = 8.2; alkalinity = 300 mg/ℓ; hardness = 360 mg/ℓ

[d] Estimated based on 24, 48, 72 and 96-hour toxicity tests

[e] Nominal concentration; because of solubility, actual concentration would be less

NR = Not reported

The marine sheepshead minnow, Cyprinodon variegatus, was relatively sensitive with a 96-hour LC_{50} value of 10.5 mg/ℓ (U.S. EPA, 1978; Heitmuller et al., 1981).

The acute toxicity of 1,2-dichlorobenzene was studied in several freshwater and marine fish (see Table 6-1). Rainbow trout, S. gairdneri, was the most sensitive species reported with an LC_{50} value of 1.67 mg/ℓ following a 96-hour exposure (Dow Chemical Company, 1978b). The U.S. EPA (1978) and Buccafusco et al. (1981) reported 96-hour LC_{50} values near 5.6 mg/ℓ for the bluegill sunfish, L. macrochirus, while Dawson et al. (1977) reported a value of 27.0 mg/ℓ for this species. The fathead minnow, P. promelas, was the most resistant species tested, having a 96-hour LC_{50} value of 57.0 mg/ℓ (Curtis et al., 1979; Curtis and Ward, 1981). Two marine species, the tidewater silverside (Menidia beryllina) and the sheepshead minnow (C. variegatus), were moderately sensitive with 96-hour LC_{50} values of 7.3 and 9.7 mg/ℓ, respectively (Dawson et al., 1977; U.S. EPA, 1978; Heitmuller et al., 1981).

The 1,3- isomer of dichlorobenzene was tested for acute toxicity in two species of freshwater fish and a single marine species. The 24, 48, 72 and 96-hour LC_{50} values for bluegill sunfish, L. macrochirus, were 21.8, 10.7, 5.02 and 5.02 mg 1,3-dichlorobenzene/ℓ, respectively (U.S. EPA, 1978; Buccafusco et al., 1981). The no-observed-effect level (NOEL) was 1.7 mg/ℓ in the bluegill (U.S. EPA, 1978). The fathead minnow, P. promelas, had a static 96-hour LC_{50} value of 12.7 mg 1,3-dichlorobenzene/ℓ (Curtis and Ward, 1981). In the marine species, sheepshead minnow (C. variegatus), 24, 48, 72 and 96-hour LC_{50} values were 8.46, 8.04, 8.04 and 7.77 mg/ℓ, respectively. The NOEL was 4.18 mg/ℓ (U.S. EPA, 1978; Heitmuller et al., 1981).

Rainbow trout, bluegill sunfish, fathead minnows and sheepshead minnows were the species tested to study the static acute toxicity of 1,4-dichlorobenzene. Rainbow trout, S. gairdneri, was the most sensitive species tested, with 48-hour LC_{50} values of 1.18 mg/ℓ (Calamari et al., 1983). The bluegill sunfish (L. macrochirus) showed 24, 48, 72 and 96-hour LC_{50} values of 4.54, 4.37, 4.37 and 4.28 mg/ℓ (U.S. EPA, 1978; Buccafusco et al., 1981). The NOEL for this species was reported to be <2.8 mg 1,4-dichlorobenzene/ℓ (U.S. EPA, 1978). The 24, 48, and 96-hour static LC_{50} values for fathead minnows (P. promelas) were 35.4, 35.4 and 33.7 mg/ℓ, respectively (Curtis et al., 1979). The marine sheepshead minnow, C. variegatus, was intermediate in sensitivity to 1,4-dichlorobenzene, having a 96-hour LC_{50} of 7.4 mg/ℓ and a NOEL of 5.6 mg/ℓ (U.S. EPA, 1978; Heitmuller et al., 1981).

1,2,4-Trichlorobenzene has been tested for acute toxicity to fish species. The 48-hour LC_{50} value for rainbow trout, S. gairdneri, was 1.95 mg/ℓ (Calamari et al., 1983). In the bluegill sunfish (L. macrochirus) estimated LC_{50}s, based on nominal concentrations, were reported for 24, 48, 72 and 96-hour exposures at 109.0, 13.0, 3.59 and 3.36 mg 1,2,4-trichlorobenzene/ℓ (U.S. EPA, 1978; Buccafusco et al., 1981). The NOEL was <1.7 mg/ℓ for the sunfish. The sheepshead minnow, C. variegatus, was more tolerant with 24, 48 and 72-hour LC_{50} values >46.8 mg/ℓ and the 96-hour LC_{50} value of 21.4 mg/ℓ. The NOEL for this marine species was 14.6 mg/ℓ (U.S. EPA, 1978; Heitmuller et al., 1981). For 1,2,3-trichlorobenzene, rainbow trout, S. gairdneri, showed a 48-hour LC_{50} value of 0.71 mg/ℓ (Calamari et al., 1983), and is thus more aquatically toxic than the 1,2,4- isomer. The corresponding LC_{50} value for Brachydanio rerio was 3.1 mg/ℓ (Calamari et al., 1983).

The toxicity of only 1,2,3,5- and 1,2,4,5-tetrachlorobenzene has been tested in fish. These two isomers differ dramatically in their lethality to bluegill sunfish and sheepshead minnows. The 24, 48, 72 and 96-hour LC_{50} values for the 1,2,3,5- isomer in bluegills (L. macrochirus) and sheepshead minnows (C. variegatus) were 57.8, 11.5, 8.34 and 6.42 mg/ℓ and >7.5, 5.59, 4.68 and 3.67 mg/ℓ, respectively (U.S. EPA, 1978; Buccafusco et al., 1981; Heitmuller et al., 1981; Ward et al., 1981). The NOELs for the bluegill and sheepshead minnow were <1.70 and 1.0 mg 1,2,3,5-tetrachlorobenzene/ℓ, respectively. The 1,2,4,5- isomer was, in some cases, 10-11 times more lethal to the fish species tested. For example, the 24, 48, 72 and 96-hour LC_{50} values in the bluegill sunfish were 5.69, 4.35, 1.55 and 1.55 mg/ℓ. In the sheepshead minnow, the LC_{50} values ranged from >1.80-0.33 for 24 through 96-hour exposures (see Table 6-1 for comparison) (U.S. EPA, 1978; Heitmuller et al., 1981; Ward et al., 1981). NOELs for the 1,2,4,5- isomer were reported to be 0.68 and 0.32 mg/ℓ for bluegill sunfish and sheepshead minnows, respectively.

The acute toxicity of pentachlorobenzene was studied in the freshwater bluegill sunfish and the marine sheepshead minnow (U.S. EPA, 1978; Buccafusco et al., 1981; Heitmuller et al., 1981). The static LC_{50} values for 24, 48, 72 and 96-hour exposures were 2.27, 0.55, 0.30 and 0.25 mg/ℓ for the bluegill sunfish (L. macrochirus) and >32.0, 9.55, 3.2-10.0 and 0.83 mg/ℓ for the sheepshead minnows (C. variegatus). NOELs for bluegill sunfish and sheepshead minnows were <0.088 and 0.32 mg pentachlorobenzene/ℓ, respectively.

Because of the low water solubility of hexachlorobenzene, acute toxicity testing of this compound has been conducted at low concentration levels.

Largemouth black bass, Micropterus salmoides, exposed for 10 days at 9-10 μg/ℓ or exposed for 15 days at 22-26 μg/ℓ, showed no toxic effects (Laska et al., 1978). Sheepshead minnows, C. variegatus, exposed at 0.13 mg/ℓ and pinfish, Lagodon rhomboides, exposed to a nominal concentration of 1.0 mg/ℓ (actual concentration would be less because of low aqueous solubility) for a 96-hour period showed no toxic effects (Parrish et al., 1974). But rainbow trout, S. gairdneri, and Brachydanio rerio showed 48-hour LC_{50} values of <0.03 mg/ℓ (Calamari et al., 1983).

Subchronic toxicity testing has been conducted on monochlorobenzene in rainbow trout, S. gairdneri (Dalich et al., 1982). Groups of fish were exposed to 2.1 or 2.9 mg monochlorobenzene/ℓ for 15 or 30 days. Treated fish did not accept food during at least the first 15 days of treatment. Neither concentration of monochlorobenzene resulted in any deaths during the exposure periods, but loss of equilibrium was reported in most treated fish. Liver toxicity, determined by enzyme levels, and histological hepatic changes were reported in trout treated at both exposure levels (Dalich et al., 1982).

Studies conducted by the U.S. EPA (1978, 1980a) resulted in chronic toxicity values (NOELs) for many of the chlorinated benzenes in fathead minnows and/or sheepshead minnows (Table 6-2).

During bioaccumulation testing with the bluegill sunfish, L. macrochirus, fish were exposed to 1,2-dichlorobenzene (7.89 μg/ℓ), 1,3-dichlorobenzene (107.0 μg/ℓ) and 1,4-dichlorobenzene (10.1 μg/ℓ) for 14 days. Similarly, 1,2,4-trichlorobenzene (2.87 μg/ℓ), 1,2,3,5-tetrachlorobenzene (7.72 μg/ℓ) and pentachlorobenzene (5.15 μg/ℓ) were tested for 28 days in the bluegill. No deaths or toxic effects were reported for any of the chlorinated benzenes at the exposure levels tested (Barrows et al., 1980).

TABLE 6-2

Chronic Toxicity Values of Chlorinated Benzenes in Fish

Chemical	Species	Chronic Value* (μg/ℓ)	Range (μg/ℓ)	Reference
1,2-Dichlorobenzene	fathead minnow (*Pimephales promelas*)	2000	1600-2500	U.S. EPA, 1978
1,3-Dichlorobenzene	fathead minnow (*Pimephales promelas*)	1510	1000-2270	U.S. EPA, 1980a
1,4-Dichlorobenzene	fathead minnow (*Pimephales promelas*)	763	560-1040	U.S. EPA, 1980a
1,2,4-Trichlorobenzene	fathead minnow (*Pimephales promelas*)	286	200-410	U.S. EPA, 1978
		705	499-995	U.S. EPA, 1980a
	sheepshead minnow (*Cyprinodon variegatus*)	222	150-330	U.S. EPA, 1978
1,2,3,4-Tetrachlorobenzene	fathead minnow (*Pimephales promelas*)	318	245-412	U.S. EPA, 1980a
1,2,4,5-Tetrachlorobenzene	sheepshead minnow (*Cyprinodon variegatus*)	129	92-180	U.S. EPA, 1978

*NOELs

Limited data are available on the pharmacokinetics of chlorinated benzenes in fish. Uptake of 1,2,4-trichlorobenzene from the water (0.012 mg/ℓ) was rapid in the rainbow trout, S. gairdneri, with bile and liver concentrations exceeding 100 times the water levels within hours (Melancon and Lech, 1980). Niimi and Cho (1980) reported that rainbow trout absorbed and accumulated hexachlorobenzene from their diet and body levels could increase 15 μg/kg body weight/day in a dose-dependent manner. Later, Oliver and Niimi (1983) reported evidence indicating that all chlorinated benzenes studied (1,2-di, 1,3-di, 1,4-di, 1,3,5-tri, 1,2,4-tri, 1,2,3-tri, 1,2,4,5-tetra, 1,2,3,4-tetra, penta- and hexachlorobenzene) could be absorbed from the aqueous environment. Zitko and Hutzinger (1976) reported the uptake and accumulation of hexachlorobenzene from food or water in juvenile Atlantic salmon, Salmo salar.

Monochlorobenzene seems to be metabolized by the liver since liver toxicity, including degeneration of hepatocytes and necrosis, was reported in treated rainbow trout (Gingerich and Dalich, 1978). A modeling study by Lu and Metcalf (1975) suggested that chlorobenzene is metabolized to o- and p-chlorophenol in the mosquito fish, Gambusia affinis.

Studies on the metabolism and biotransformation of 1,2,4-trichlorobenzene in rainbow trout (S. gairdneri) and carp (Cyprinus carpio) suggested that conjugated metabolites occur in the liver and bile (Melancon and Lech, 1980). A hepatic mixed-function oxidase inducer (β-naphthoflavone) elevates the hepatic and biliary levels of biotransformation products of 1,2,4-trichlorobenzene. In the mosquito fish, G. affinis, absorbed hexachlorobenzene is predominantly unchanged, but two unidentified metabolites were reported (Lu and Metcalf, 1975).

Accumulated chlorinated benzenes and/or their metabolites seem to be distributed throughout the body in fish. The highest concentrations have been detected in the bile, liver and muscle (Melancon and Lech, 1980). The bioconcentration of chlorinated benzenes increased as the degree of chlorination of the test compound increased (Oliver and Niimi, 1983). Bioconcentration factors (BCFs) for many of the chlorinated benzenes in guppies (_Poecilia_ _reticulata_) and rainbow trout (_S_. _gairdneri_) are shown in Table 6-3 (Konemann and van Leeuwen, 1980; Oliver and Niimi, 1983). More complete data on BCFs in fish are reported in Section 5.3 of this document.

The excretion rate of chlorinated benzenes in fish is related to the extent of chlorination of the compound. Konemann and van Leeuwen (1980) reported that 1,4-dichlorobenzene is excreted within several days, while trichlorobenzenes required nearly 25 days, tetrachlorobenzene nearly 50 days, and penta- and hexachlorobenzene required >50 days for elimination. After termination of exposure, 1,2,4-trichlorobenzene and metabolites are eliminated in two stages. The first had a half-life of elimination of 0.4 days, while the second was eliminated more slowly ($t_{1/2}$ = 50 days). In comparison, Branson et al. (1975) reported half-lives for elimination of dichlorobenzene and hexachlorobenzene to be 1.1 and 12.1 days, respectively. Sanborn et al. (1977) estimated the half-life for elimination of hexachlorobenzene in the green sunfish, _L_. _cyanellus_, to be 8.0-19.6 days. The longest time was for elimination from the liver. The biological half-life of hexachlorobenzene was estimated to be 7 months to several years in rainbow trout (Niimi and Cho, 1981).

6.1.2. Effect on Aquatic Crustaceans. In addition to fish, freshwater and marine crustaceans, which are an important element in aquatic food chains, are exposed to chlorobenzenes in the environment (Grzenda et al.,

TABLE 6-3

Bioconcentration Factors of Some Chlorinated Benzenes in Two Fish Species

Species	Compound	Exposure Level (μg/ℓ)	BCF	Reference
Rainbow trout *Salmo gairdneri*	1,2-di-	47.0	270	Oliver and Niimi, 1983
		940.0	560	
	1,3-di-	28.0	420	
		690.0	740	
	1,4-di-	28.0	370	
		670.0	720	
	1,3,5-tri-	2.3	1,800	
		45.0	4,100	
	1,2,4-tri-	3.2	1,300	
		52.0	3,200	
	1,2,3-tri-	4.3	1,200	
		72.0	2,600	
	1,2,4,5-tetra-	1.0	5,300	
		21.0	13,000	
	1,2,3,4-tetra-	1.4	5,200	
		26.0	12,000	
	penta-	0.34	13,000	
		9.3	20,000	
	hexa-	0.32	12,000	
		8.0	20,000	
Guppy *Poecilia reticulata*	1,4-di-	116.0	1,800	Konemann and van Leeuwen, 1980
	1,2,3-tri-	48.0	13,000	
	1,3,5-tri-	43.0	14,000	
	1,2,3,5-tetra-	12.0	72,000	
	penta-	1.2	260,000	
	hexa-	0.3	290,000	

1964). Laboratory testing of the chlorinated benzenes has provided acute toxicity data for several species of crustaceans (Table 6-4).

The U.S. EPA (1978) reported most of the available data in which mono-, 1,2-di-, 1,3-di-, 1,4-di-, 1,2,4-tri-, 1,2,3,5-tetra-, 1,2,4,5-tetra- and pentachlorobenzene toxicities were tested in the water flea (Daphnia magna) and the mysid shrimp (Mysidopsis bahia). Other available studies on specific chlorinated benzenes tested in specific species were noted in Table 6-2. Generally, the more chlorinated benzenes appear to be more toxic. For example, the 96-hour LC_{50} values in mysid shrimp were 16.4, 1.97, 0.34 and 0.16 mg/ℓ for mono-, 1,2-di-, 1,2,3,5-tetra- and pentachlorobenzene, respectively. Data on the toxicity of the 1,2,3,5- and 1,2,4,5-tetrachlorobenzenes indicate that, unlike that in fish, the 1,2,3,5- isomer is more toxic. Because of the very low solubility of hexachlorobenzene in aqueous solutions, data on the toxicity of this compound are limited. One study (Laska et al., 1978) reported no toxic effects in crayfish, Procambarus clarkii, exposed (unspecified interval) to a saturated aqueous solution of hexachlorobenzene (estimated to be ~0.02 mg/ℓ). The 24-hour immobilization concentrations of several chlorobenzenes for water fleas, Daphnia magna, using the AFNOR test were: monochlorobenzene (4.3 mg/ℓ); 1,2-dichlorobenzene (0.78 mg/ℓ); 1,4-dichlorobenzene (<0.03 mg/ℓ) (Calamari et al., 1983).

6.1.3. Embryotoxic and Reproductive Effects. Wild Atlantic salmon (Salmo salar) eggs, collected from different sites, contained different levels of hexachlorobenzene (0.086, 0.132, 0.142 and 0.159 μg/g lipid in eggs). No correlation between hexachlorobenzene levels and egg-hatchability was demonstrated (Zitko and Saunders, 1979). Eggs also contained other environmental contaminants such as PCBs and organochloride pesticides.

TABLE 6-4

Acute Toxicity Data for Crustaceans Exposed to Chlorinated Benzenes

Compound	Species	Duration (hour)	Mean Concentration (mg/ℓ)	Method	Effect	Reference
Monochlorobenzene	water flea (*Daphnia magna*)	24	140.0	static	LC_{50}	U.S. EPA, 1978; LeBlanc, 1980
		48	86.0	static	LC_{50}	
		48	10.0	static	None	
		24	4.3	AFNOR	IC_{50}	Calamari et al., 1983
	mysid shrimp (*Mysidopsis bahia*)	24	24.7	static	LC_{50}	U.S. EPA, 1978
		48	24.7	static	LC_{50}	U.S. EPA, 1978
		72	24.7	static	LC_{50}	U.S. EPA, 1978
		96	16.4	static	LC_{50}	U.S. EPA, 1978
		96	<11.1	static	None	U.S. EPA, 1978
1,2-Dichlorobenzene	water flea (*Daphnia magna*)	24	2.44	static	LC_{50}	U.S. EPA, 1978; LeBlanc, 1980
		48	2.44	static	LC_{50}	
		48	0.36	static	None	
		24	0.78	AFNOR	IC_{50}	Calamari et al., 1983
	mysid shrimp (*Mysidopsis bahia*)	24	4.75	static	LC_{50}	U.S. EPA, 1978
		48	4.52	static	LC_{50}	U.S. EPA, 1978
		72	3.88	static	LC_{50}	U.S. EPA, 1978
		96	1.97	static	LC_{50}	U.S. EPA, 1978
		96	<1.29	static	None	U.S. EPA, 1978
	grass shrimp (*Palaemonetes pugio*)	24	14.3	static	LC_{50}	Curtis et al., 1979
		48	10.3	static	LC_{50}	Curtis et al., 1979
		96	9.4	static	LC_{50}	Curtis et al., 1979
		96	10.4	static	LC_{50}	Curtis and Ward, 1981
1,3-Dichlorobenzene	water flea (*Daphnia magna*)	24	47.8	static	LC_{50}	U.S. EPA, 1978; LeBlanc, 1980
		48	28.1	static	LC_{50}	
		48	6.0	static	None	
	mysid shrimp (*Mysidopsis bahia*)	24	7.31-13.06	static	LC_{50}	U.S. EPA, 1978
		48	5.14	static	LC_{50}	U.S. EPA, 1978
		72	4.06	static	LC_{50}	U.S. EPA, 1978
		96	2.85	static	LC_{50}	U.S. EPA, 1978
		96	<1.30	static	None	U.S. EPA, 1978

TABLE 6-4 (cont.)

Compound	Species	Duration (hour)	Mean Concentration (mg/ℓ)	Method	Effect	Reference
1,4-Dichlorobenzene	water flea (*Daphnia magna*)	24	41.5	static	LC_{50}	U.S. EPA, 1978; LeBlanc, 1980
		48	11.0	static	LC_{50}	
		48	0.68	static	None	
		24	1.6	AFNOR	IC_{50}	Calamari et al., 1983
	mysid shrimp (*Mysidopsis bahia*)	24	5.6-10.0	static	LC_{50}	U.S. EPA, 1978
		48	5.35	static	LC_{50}	U.S. EPA, 1978
		72	4.31	static	LC_{50}	U.S. EPA, 1978
		96	1.99	static	LC_{50}	U.S. EPA, 1978
		96	<1.0	static	None	U.S. EPA, 1978
	grass shrimp (*Palaemonetes pugio*)	48	129.2	static	LC_{50}	Curtis et al., 1979
		96	69.0	static	LC_{50}	Curtis et al., 1979
		96	60.0	static	LC_{50}	Curtis and Ward, 1981
1,2,3-Trichlorobenzene	water flea (*Daphnia magna*)	24	0.35	AFNOR	IC_{50}	Calamari et al., 1983
1,2,4-Trichlorobenzene	water flea (*Daphnia magna*)	24	114.0	static	LC_{50}	U.S. EPA, 1978; LeBlanc, 1980
		48	50.2	static	LC_{50}	
		48	<2.4	static	None	
		24	1.2	AFNOR	IC_{50}	Calamari et al., 1983
	mysid shrimp (*Mysidopsis bahia*)	24	>1.46	static	LC_{50}	U.S. EPA, 1978
		48	>1.46	static	LC_{50}	U.S. EPA, 1978
		72	0.76	static	LC_{50}	U.S. EPA, 1978
		96	0.45	static	LC_{50}	U.S. EPA, 1978
		96	0.09	static	None	U.S. EPA, 1978
1,3,5-Trichlorobenzene	brine shrimp (*Artemia salina*)	168	10.0	static	LC_{100}	Grosch, 1973
1,2,3,5-Tetrachlorobenzene	water flea (*Daphnia magna*)	24	18.1	static	LC_{50}	U.S. EPA, 1978; LeBlanc, 1980
		48	9.71	static	LC_{50}	
		48	<1.1	static	None	
	mysid shrimp (*Mysidopsis bahia*)	24	0.96	static	LC_{50}	U.S. EPA, 1978
		48	0.36	static	LC_{50}	U.S. EPA, 1978
		72	0.34	static	LC_{50}	U.S. EPA, 1978
		96	0.34	static	LC_{50}	U.S. EPA, 1978
		96	0.10	static	None	U.S. EPA, 1978

TABLE 6-4 (cont.)

Compound	Species	Duration (hour)	Mean Concentration (mg/ℓ)	Method	Effect	Reference
1,2,4,5-Tetrachlorobenzene	water flea (*Daphnia magna*)	24	>530.0	static	LC_{50}	U.S. EPA, 1978; LeBlanc, 1980
		48	>530.0	static	LC_{50}	
		48	320.0	static	None	
	mysid shrimp (*Mysidopsis bahia*)	24	3.2-5.6	static	LC_{50}	U.S. EPA, 1978
		48	1.99	static	LC_{50}	U.S. EPA, 1978
		72	1.48	static	LC_{50}	U.S. EPA, 1978
		96	1.48	static	LC_{50}	U.S. EPA, 1978
		96	0.6	static	None	U.S. EPA, 1978
Pentachlorobenzene	water flea (*Daphnia magna*)	24	17.2	static	LC_{50}	U.S. EPA, 1978; LeBlanc, 1980
		48	5.28	static	LC_{50}	
		48	1.3	static	None	
	mysid shrimp (*Mysidopsis bahia*)	24	0.75	static	LC_{50}	U.S. EPA, 1978
		48	0.72	static	LC_{50}	U.S. EPA, 1978
		72	0.24	static	LC_{50}	U.S. EPA, 1978
		96	0.16	static	LC_{50}	U.S. EPA, 1978
		96	<0.06	static	None	U.S. EPA, 1978
Hexachlorobenzene	water flea (*Daphnia magna*)	24	<0.03	AFNOR	IC_{50}	Calamari et al., 1983
	swamp crayfish (*Procambarus clarkii*)	NR*	saturated*	static or flowthrough*	No toxic effects	Laska et al., 1978
	shrimp (*Crangon septemspinosa*)	96	0.0072	static	No mortality	McLeese and Metcalfe, 1980

*Toxicity testing was conducted for an unspecified period with a saturated aqueous solution of hexachlorobenzene in both static and flowthrough systems.

NR = Not reported

LC_{50} = Lethal concentration for 50% of animals; IC_{50} = immobilization concentration for 50% of animals

The toxic effects of monochlorobenzene on egg and embryo development were studied in the laboratory with largemouth bass (Micropterus salmoides), goldfish (Carassius auratus) and rainbow trout (Salmo gairdneri) using a flowthrough system with both hard (200 mg/ℓ $CaCO_3$) and soft (50 mg/ℓ $CaCO_3$) water (Birge et al., 1979). With trout, exposure to 0.09, 0.31, 1.60, 4.27 and 32.0 mg monochlorobenzene/ℓ was initiated 20 minutes after fertilization and continued for 16 days (hatching time for trout is 23 days). Complete lethality of the trout embryos occurred at all monochlorobenzene concentrations within the exposure period in hard and soft water conditions (Table 6-5). The LC_{50} for trout embryos was therefore reported to be <0.09 mg/ℓ (Birge et al., 1979). Largemouth bass embryos/larvae were exposed 1-2 hours postfertilization through hatching until 4 days posthatching. (Average hatching time for bass is 3.5 days.) Chlorobenzene concentrations ranged from 0.013-27.3 mg/ℓ for soft water and 0.009-23.2 mg/ℓ for hard water conditions. Percent hatchability was reduced to 72, 25 and 4% of controls at 0.15, 3.10 and 23.2 mg/ℓ, respectively, in hard water. Percent survival of bass larvae at 4 days posthatching was 80, 60 and 24% after exposure to 0.013, 0.038 and 0.16 mg monochlorobenzene/ℓ, respectively, in soft water conditions. The LC_{50} value at 4 days posthatching for bass larvae was reported to be 0.05-0.06 mg/ℓ, while the LC_{50} value for embryos exposed until hatching was 0.34-0.39 mg/ℓ (see Table 6-5). Goldfish, C. auratus, were more tolerant to monochlorobenzene exposure during development. (Average hatching time for goldfish is 4 days.) The LC_{50} values for embryos exposed until hatching and embryos/larvae exposed until 4 days post-hatching ranged from 2.37-3.48 mg/ℓ and 0.88-1.04 mg/ℓ, respectively (see Table 6-5). Abnormal bass larvae

TABLE 6-5

Embryo-Larval Toxicity of Monochlorobenzene to Goldfish, Largemouth Bass and Rainbow Trout in Soft and Hard Water[a]

		Soft Water (50 mg/ℓ as $CaCO_3$)		Hard Water (200 mg/ℓ as $CaCO_3$)	
Species	Exposure in Days Beyond Egg Hatching	LC_{50} (mg/ℓ)	95% Confidence Limits	LC_{50} (mg/ℓ)	95% Confidence Limits
Goldfish[b]	0	3.48	3.08-3.87	2.37	1.96-2.86
	4	0.88	0.67-1.12	1.04	0.86-1.25
Largemouth bass[c]	0	0.34	0.22-0.51	0.39	0.25-0.58
	4	0.05	0.04-0.07	0.06	0.04-0.08
Rainbow trout[d]	c/	<0.09	NA	<0.09	NA

[a] Source: Birge et al., 1979

[b] Require ~4 days from spawning to hatching; thus, exposure of the hatched larvae for 4 additional days resulted in a total of 8 days of continuous exposure.

[c] Require ~3.5 days from spawning to hatching; thus, exposure of the hatched larvae for 4 additional days resulted in a total of 7.5 days of continuous exposure.

[d] Require ~23 days from spawning to hatching; all exposed embryos were dead by 16 days after fertilization.

NA = Not applicable

occurred in 2, 13, 42 and 100% of those hatching after exposure to 0.04, 0.15, 3.1 and 23.2 mg/ℓ, respectively, during embryonic development. Abnormal goldfish larvae were less prevalent (Birge et al., 1979).

The embryo and larval toxicity of 1,2,4,5-tetrachlorobenzene was tested in sheepshead minnows, C. variegatus. Within 4 hours after assurance of fertilization, embryos were exposed to 0.06, 0.09, 0.18, 0.30 and 0.52 mg 1,2,4,5-tetrachlorobenzene/ℓ until hatching; thereafter, exposure of larval and juvenile fish was continued for an additional 28 days. Hatching success of embryos was not significantly decreased at any exposure level. Juvenile mortality was significantly ($p<0.05$) increased in fish exposed to >0.18 mg 1,2,4,5-tetrachlorobenzene/ℓ (Table 6-6). The maximum acceptable toxicant concentration (MATC) for embryos and juvenile sheepshead minnows exposed to 1,2,4,5-tetrachlorobenzene was estimated to range between 0.09-0.18 mg/ℓ.

The embryo and larval toxicity of trichlorobenzene (isomer not specified) was studied in American oysters (Crassostrea virginica) and the hard clam (Mercenaria mercenaria) (Davis and Hindu, 1969). Exposure which commenced soon after fertilization and embryo development was determined 48 hours later. To determine larval survival, 2-day-old larvae (hatched under normal conditions) were exposed for 10 days (for clams) or 12 days (for oysters) before quantitative sampling. At 1.0 and 10.0 mg trichlorobenzene/ℓ, egg survival and normal embryo development in oysters was 59 and 21%, respectively, of control cultures. In clams treated with 1.0 and 10.0 mg trichlorobenzene/ℓ, embryo development was reduced to 72 and 58% of controls. Survival of clam larvae exposed to 1.0 and 10.0 mg trichlorobenzene/ℓ was 108 and 69% of controls, respectively, with no change in larval length. Based on toxicity data, Davis and Hindu (1969) reported a 48-hour

TABLE 6-6

Results of 1,2,4,5-Tetrachlorobenzene Tests with Embryo to Juvenile Sheepshead Minnows in Continuous-Flow Natural Seawater[a]

Nominal Concentration (mg/ℓ)	Measured Concentration[b] (mg/ℓ)	Hatching Success (%)	Juvenile Mortality[c] (%)	Standard Length of Juveniles (mm)
Control	ND	84	21	11±2
Solvent control	ND	85	25	12±3
0.12	0.06±0.04	76	16	10±3
0.25	0.09±0.04	81	41	12±2
0.5	0.18±0.07	91	54[d]	10±3
1.0	0.30±0.16	83	79[d]	12±1
2.0	0.52±0.33	67	98[d]	12±0[e]

[a] Source: Ward et al., 1981

[b] Values expressed as mean ± standard deviation

[c] At 28 days after hatching

[d] Significantly greater than control at $p<0.05$

[e] Only one fish survived: the 96-hour LC_{50} for juveniles was 0.33 mg/ℓ with 95% confidence limits of 0.12-0.94 mg/ℓ.

ND = Not detectable (<0.007 mg/ℓ)

LC_{50} of 3.13 mg/ℓ for oyster embryos and 48-hour and 12-day LC_{50} values of >10.0 mg/ℓ for clam embryos and larvae.

The effects of 1,3,5-trichlorobenzene on the reproductive performance in brine shrimp, _Artemia salina_, were reported by Grosch (1973). Ten pairs of adult shrimp were exposed to 10 ppm 1,3,5-trichlorobenzene for 24 hours and studied for their lifetime for reproductive performance. The lifespan of treated adult females was significantly ($p<0.05$) reduced. The number of broods, number of zygotes, and larval survival rate were all significantly reduced in exposed cultures (Table 6-7). The author discussed the possibility that brood number and zygote number were related to the decreased lifespan of adult females, but discounted this as the sole cause after computations showed a decrease in the brood size (Grosch, 1973). Cultures of _Artemia salina_ that were continuously exposed to 10 mg 1,3,5-trichlorobenzene/ℓ survived <1 week and produced no viable embryos.

6.1.4. **Effect on Aquatic Plants.** The 96-hour EC_{50} (effective concentration for 50% of the algae to show the effect) for reduced chlorophyll _a_ content in the freshwater algae, _Selenastrum capricornutum_, treated with monochlorobenzene was 232 mg/ℓ (Table 6-8). The 96-hour EC_{50} for inhibition of growth and the reported NOEL were 224 and <111 mg/ℓ, respectively (U.S. EPA, 1978). (For more complete toxicity data for algae refer to Table 6-8.). Toxicity of 1,2-, 1,3- and 1,4-dichlorobenzene was somewhat varied when comparing 96-hour EC_{50} values for reduced chlorophyll content of 91.6, 179 and 98.1, respectively. The general trend of increasing toxicity with increased chlorine substitution is seen with 1,2,4-trichlorobenzene, tetrachlorobenzenes and pentachlorobenzene (see Table 6-8). The 1,2,3,5- isomer of tetrachlorobenzene appears to be 2- to 3-fold more toxic than the 1,2,4,5- isomer in this freshwater algae, _S_. _capricornutum_. The

TABLE 6-7

Adult Lifespan and Reproductive Performance of Brine Shrimp Exposed to 1,3,5-Trichlorobenzene[a,b]

	Brine Solution Controls	Acetone Controls	Exposed to 1,3,5-Trichlorobenzene
Adults:			
Survival (in days)			
Males[c]	49.6±4.0	47.6±4.0	44.2±3.8
Females[d]	50.0±5.0	50.1±5.5	37.6±4.2
Number of broods (per pair)[e]	11.3±1.6	11.8±1.6	5.3±0.8
Offspring:			
Total number of zygotes produced[f]	1828	1884	456
Cysts produced (%)[f]	29.0	30.6	11.4
Cysts hatched (%)[f]	46±5	48±7	18±10
Survival of larvae (%)[f]	76.3±5.0	75.6±4.7	30.3±11.5
Sex ratio (no. males/no. females)[f]	0.91	0.94	0.82
Adaptive values (ratio of average no. of matured offspring per pair exposed to 1,3,5-trichlorobenzene/average no. of matured offspring per pair in acetone controls)[f]		1.00	0.11

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[a] Source: Grosch, 1973

[b] Tests performed with 10 mating pairs exposed at 10 mg/ℓ for 24 hours; each pair then returned to separate fresh brine solutions.

[c] Control and treatment means not statistically different.

[d] Statistically significant difference between control and treatment means at 0.05 level.

[e] Statistically significant difference between control and treatment means at 0.005 level.

[f] Statistical analyses not reported for difference between control and treatment means.

TABLE 6-8

Acute Toxicity Data for Aquatic Algae Exposed to Chlorinated Benzenes

Compound	Species	Duration (hours)	Mean Concentration (mg/ℓ)	Method	Effect	Reference
Monochlorobenzene	freshwater algae (*Selenastrum capricornutum*)	24[a]	330.0	static	EC_{50}	U.S. EPA, 1978
		48[a]	264.0	static	EC_{50}	U.S. EPA, 1978
		96[a]	232.0	static	EC_{50}	U.S. EPA, 1978
		96[b]	224.0	static	EC_{50}	U.S. EPA, 1978
		96[c]	<111.0	static	None	U.S. EPA, 1978
		96	12.5	static	EC_{50}	Calamari et al., 1983
	marine algae (*Skeletonema costatum*)	24[a]	263.0	static	EC_{50}	U.S. EPA, 1978
		48[a]	224.0	static	EC_{50}	U.S. EPA, 1978
		96[a]	343.0	static	EC_{50}	U.S. EPA, 1978
		96[b]	341.0	static	EC_{50}	U.S. EPA, 1978
		96[c]	<221.0	static	None	U.S. EPA, 1978
	Green algae (*Scendesmus quadricauda*)	168	>390.0	static	EC_3[d]	Bringmann and Kuhn, 1980
1,2,-Dichlorobenzene	freshwater algae (*Selenastrum capricornutum*)	24[a]	84.8	static	EC_{50}	U.S. EPA, 1978
		48[a]	138.0	static	EC_{50}	U.S. EPA, 1978
		72[a]	119.0	static	EC_{50}	U.S. EPA, 1978
		96[a]	91.6	static	EC_{50}	U.S. EPA, 1978
		96[b]	98.0	static	EC_{50}	U.S. EPA, 1978
		96[c]	<12.9	static	None	U.S. EPA, 1978
		96	2.2	static	EC_{50}	Calamari et al., 1983
	marine algae (*Skeletonema costatum*)	24[a]	66.7	static	EC_{50}	U.S. EPA, 1978
		48[a]	45.1	static	EC_{50}	U.S. EPA, 1978
		72[a]	45.6	static	EC_{50}	U.S. EPA, 1978
		96[a]	44.2	static	EC_{50}	U.S. EPA, 1978
		96[b]	44.1	static	EC_{50}	U.S. EPA, 1978
		96[c]	<12.8	static	None	U.S. EPA, 1978
	Green algae (*Scendesmus quadricauda*)	168	>100.0	static	EC_3[d]	Bringmann and Kuhn, 1980
1,3-Dichlorobenzene	freshwater algae (*Selenastrum capricornutum*)	24[a]	180.0	static	EC_{50}	U.S. EPA, 1978
		48[a]	170.0	static	EC_{50}	U.S. EPA, 1978
		72[a]	162.0	static	EC_{50}	U.S. EPA, 1978
		96[a]	179.0	static	EC_{50}	U.S. EPA, 1978
		96[b]	149.0	static	EC_{50}	U.S. EPA, 1978
		96[c]	41.8	static	None	U.S. EPA, 1978

TABLE 6-8 (cont.)

Compound	Species	Duration (hours)	Mean Concentration (mg/ℓ)	Method	Effect	Reference
1,3-Dichlorobenzene (cont.)	marine algae (Skeletonema costatum)	24^a	55.8	static	EC_{50}	U.S. EPA, 1978
		48^a	41.9	static	EC_{50}	U.S. EPA, 1978
		72^a	62.3	static	EC_{50}	U.S. EPA, 1978
		96^a	52.8	static	EC_{50}	U.S. EPA, 1978
		96^b	49.6	static	EC_{50}	U.S. EPA, 1978
		96^c	7.3	static	None	U.S. EPA, 1978
1,4-Dichlorobenzene	freshwater algae (Selenastrum capricornutum)	24^a	76.9	static	EC_{50}	U.S. EPA, 1978
		48^a	61.6	static	EC_{50}	U.S. EPA, 1978
		72^a	77.5	static	EC_{50}	U.S. EPA, 1978
		96^a	98.1	static	EC_{50}	U.S. EPA, 1978
		96^b	96.7	static	EC_{50}	U.S. EPA, 1978
		96^c	5.6	static	None	U.S. EPA, 1978
		96	1.6	static	EC_{50}	Calamari et al., 1983
	marine algae (Skeletonema costatum)	24^a	61.9	static	EC_{50}	U.S. EPA, 1978
		48^a	56.6	static	EC_{50}	U.S. EPA, 1978
		72^a	50.6	static	EC_{50}	U.S. EPA, 1978
		96^a	54.8	static	EC_{50}	U.S. EPA, 1978
		96^b	59.1	static	EC_{50}	U.S. EPA, 1978
		96^c	10.0	static	None	U.S. EPA, 1978
1,2,3-Trichlorobenzene	freshwater algae (Selenastrum capricornutum)	96	0.9	static	EC_{50}	Calamari et al., 1983
1,2,4-Trichlorobenzene	freshwater algae (Selenastrum capricornutum)	24^a	55.0	static	EC_{50}	U.S. EPA, 1978
		48^a	32.8	static	EC_{50}	U.S. EPA, 1978
		72^a	31.8	static	EC_{50}	U.S. EPA, 1978
		96^a	35.3	static	EC_{50}	U.S. EPA, 1978
		96^b	36.7	static	EC_{50}	U.S. EPA, 1978
		96^c	<8.2	static	None	U.S. EPA, 1978
		96	1.4	static	EC_{50}	Calamari et al., 1983
	marine algae (Skeletonema costatum)	24^a	13.5	static	EC_{50}	U.S. EPA, 1978
		48^a	1.46-2.63	static	EC_{50}	U.S. EPA, 1978
		72^a	1.46-2.63	static	EC_{50}	U.S. EPA, 1978
		96^a	8.75	static	EC_{50}	U.S. EPA, 1978
		96^b	8.93	static	EC_{50}	U.S. EPA, 1978
		96^c	<1.46	static	None	U.S. EPA, 1978

TABLE 6-8 (cont.)

Compound	Species	Duration (hours)	Mean Concentration (mg/ℓ)	Method	Effect	Reference
1,2,3,5-Tetrachlorobenzene	freshwater algae (*Selenastrum capricornutum*)	24^a	27.4	static	EC_{50}	U.S. EPA, 1978
		48^a	28.0	static	EC_{50}	U.S. EPA, 1978
		72^a	14.7	static	EC_{50}	U.S. EPA, 1978
		96^a	17.2	static	EC_{50}	U.S. EPA, 1978
		96^b	17.7	static	EC_{50}	U.S. EPA, 1978
		96^c	<3.2	static	None	U.S. EPA, 1978
	marine algae (*Skeletonema costatum*)	24^a	2.83	static	EC_{50}	U.S. EPA, 1978
		48^a	2.53	static	EC_{50}	U.S. EPA, 1978
		72^a	1.39	static	EC_{50}	U.S. EPA, 1978
		96^a	0.83	static	EC_{50}	U.S. EPA, 1978
		96^b	0.70	static	EC_{50}	U.S. EPA, 1978
		96^c	<0.1	static	None	U.S. EPA, 1978
1,2,4,5-Tetrachlorobenzene	freshwater algae (*Selenastrum capricornutum*)	24^a	50.4	static	EC_{50}	U.S. EPA, 1978
		48^a	54.9	static	EC_{50}	U.S. EPA, 1978
		72^a	47.3	static	EC_{50}	U.S. EPA, 1978
		96^a	52.9	static	EC_{50}	U.S. EPA, 1978
		96^b	46.8	static	EC_{50}	U.S. EPA, 1978
		96^c	<3.2	static	None	U.S. EPA, 1978
	marine algae (*Skeletonema costatum*)	24^a	>18.0	static	EC_{50}	U.S. EPA, 1978
		48^a	9.39	static	EC_{50}	U.S. EPA, 1978
		72^a	8.56	static	EC_{50}	U.S. EPA, 1978
		96^a	7.10	static	EC_{50}	U.S. EPA, 1978
		96^b	7.32	static	EC_{50}	U.S. EPA, 1978
		96^c	<1.0	static	None	U.S. EPA, 1978
Pentachlorobenzene	freshwater algae (*Selenastrum capricornutum*)	24^a	>32.0	static	EC_{50}	U.S. EPA, 1978
		48^a	8.25	static	EC_{50}	U.S. EPA, 1978
		72^a	13.0	static	EC_{50}	U.S. EPA, 1978
		96^a	6.78	static	EC_{50}	U.S. EPA, 1978
		96^b	6.63	static	EC_{50}	U.S. EPA, 1978
		96^c	0.10	static	None	U.S. EPA, 1978
	marine algae (*Skeletonema costatum*)	24^a	5.53	static	EC_{50}	U.S. EPA, 1978
		48^a	1.57	static	EC_{50}	U.S. EPA, 1978
		72^a	1.94	static	EC_{50}	U.S. EPA, 1978
		96^a	2.23	static	EC_{50}	U.S. EPA, 1978
		96^b	1.98	static	EC_{50}	U.S. EPA, 1978
		96^c	<0.1	static	None	U.S. EPA, 1978

TABLE 6-8 (cont.)

Compound	Species	Duration (hours)	Mean Concentration (mg/ℓ)	Method	Effect	Reference
Hexachlorobenzene	Freshwater algae (*Selenastrum capricornutum*)	96	<0.03	static	EC_{50}	Calamari et al., 1983
	Tetrahymena pyriformis	240	0.001	static	Growth reduction[e]	Geike and Parasher, 1976
	Mixed culture; diatom/green algae (*Thalassiosira pseudonana*/*Dunaliella tertiolecta*)	72	0.1	static	No growth inhibition	Biggs et al., 1979
	Green algae (*Chlorella pyrenoidosa*)	76	10.0	static	Growth reduction[f]	Parasher et al., 1978

[a] Effective on chlorophyll *a* content

[b] Effective on cell growth

[c] NOEL

[d] A 3% change in growth measured by turbidity

[e] Growth reduced to 66% of control cultures; measured by dry mass

[f] Growth reduced to 87.5% of control cultures; measured by dry mass

EC_{50} = Concentration inhibiting the growth of 50% of the population

U.S. EPA (1978) also conducted similar toxicity tests on the chlorinated benzenes with the marine algae, Skeletonema costatum. The 24, 48, 72 and 96-hour EC_{50} values and the 96-hour NOELs for the chlorinated benzenes studied are shown in Table 6-8. Effective toxicity concentrations of each chlorinated benzene are within the same range for both the freshwater and marine algae. Data from other studies (Bringmann and Kuhn, 1980; Geike and Parasher, 1976; Biggs et al., 1979; Parasher et al., 1978) using various algal species are also reported in Table 6-8.

6.1.5. Residues. Residue concentrations of the chlorinated benzenes (in sediment and water) were determined in the Great Lakes (Superior, Huron, Erie and Ontario), drinking water of surrounding cities, wastewater effluents from area industries and from the Grand and Niagara Rivers (Oliver and Nicol, 1982). These data, reviewed in Table 6-9, indicate that almost all chlorinated benzenes exist in measurable quantities in the Great Lakes and can occasionally be traced to point sources. Oliver and Nicol (1982) indicate that these substances are persistent in the sediment and are bioconcentrated by fish.

Bjerk and Brevik (1980) collected sediment core samples (0-5 cm deep) and reported concentrations of 0.87 mg/kg pentachlorobenzene and 0.528 mg/kg hexachlorobenzene (dry weight basis) in the Oslo fjord at Asstranda, Norway. Deeper samples contained less contaminants (0.064 and 0.317 mg/kg, respectively). At Ora, Norway, sediment samples contained lower levels of pentachlorobenzene (0.003 mg/kg dry weight). In a wide variety of species tested (algae, crustaceans, mollusks and fish), penta- and hexachlorobenzene appear to bioaccumulate, usually about 20-fold over environmental levels (Table 6-10).

TABLE 6-9

Chlorinated Benzene Concentrations (µg/ℓ) in Water and Sediment[a]

Chemical	Lake Superior	Lake Huron	Lake Erie	Lake Ontario	City Drinking Water	Wastewater Effluents	Niagara[b] River	Grand River	Sample Type
1,3-Dichlorobenzene	NA	ND	NA	ND	1	14	18	1	W
	2	2	4	74	NA	NA		NA	S
1,4-Dichlorobenzene	NA	4	NA	45	13	660	94	10	W
	5	16	9	94	NA	NA		NA	S
1,2-Dichlorobenzene	NA	ND	NA	5	3	13	56	6	W
	1	8	2	11	NA	NA		NA	S
1,3,5-Trichlorobenzene	NA	ND	NA	0.1	ND	0.3	8	ND	W
	0.2	0.7	1	60	NA	NA		NA	S
1,2,4-Trichlorobenzene	NA	0.2	NA	0.6	2	11	107	2	W
	1	6	3	94	NA	NA		NA	S
1,2,3-Trichlorobenzene	NA	ND	NA	0.1	0.1	2	38	0.1	W
	0.2	0.3	0.4	7	NA	NA		NA	S
1,2,3,5-Tetrachlorobenzene	NA	ND	NA	ND	ND	0.4	3	ND	W
	0.1	0.4	0.3	6	NA	NA		NA	S
1,2,4,5-Tetrachlorobenzene	NA	ND	NA	0.1	0.2	1.2	31	ND	W
	0.3	1	1	52	NA	NA		NA	S
1,2,3,4-Tetrachlorobenzene	NA	0.05	NA	0.1	0.3	1.6	126	0.05	W
	0.3	1	0.7	33	NA	NA		NA	S
Pentachlorobenzene	NA	0.04	NA	0.2	0.04	0.9	22	0.05	W
	0.1	1	1	32	NA	NA		NA	S
Hexachlorobenzene	NA	0.04	NA	0.06	0.1	1.5	17	0.06	W
	0.2	2	3	97	NA	NA		NA	S

[a] Source: Oliver and Nicol, 1982

[b] Highest value of four sampling sites reported

NA = Not available; ND = Not dectected; S = Mean concentration in surficial sediment sample; W = Mean concentration in water samples

TABLE 6-10

Chlorinated Benzene Concentrations in a Variety of Marine Species

Species/Tissue	Number Analyzed	Mean Concentration (mg/kg) of Chlorinated Benzene Tri-	Tetra-	Penta-	Hexa-	Reference
Cod (*Gadus morhua*)	7	0.4	0.3	3.8	55.6	Ofstad et al., 1978
Cod, homogenate	6	NA	NA	0.79	19.9	Bjerk and Brevik, 1980
Cod liver	6	NA	NA	NA	30.9	Bjerk and Brevik, 1980
Cod liver	3	2.7	0.8	12.7	170	Ofstad et al., 1978
Cod fillet	3	0.4	0.14	1.1	31	Ofstad et al., 1978
Whiting	2	1.1	0.3	4.3	56	Ofstad et al., 1978
Sprat	4	0.5	0.3	4.7	29	Ofstad et al., 1978
Sprat oil^a		<0.01-0.5	<0.01-0.4	0.01-3.7	0.04-16	Lunde and Ofstad, 1976
Plaice	3	0.2	0.4	0.7	13	Ofstad et al., 1978
Eel	3	0.3	0.3	0.7	13	Ofstad et al., 1978
Rainbow trout (*Salmo gairdneri*)	10	0.6	1.5	3.5	32.7	Oliver and Niimi, 1983
Brown trout (*Salmo trutta*)	6	NA	NA	NA	31.7	Skaftason and Johannesson, 1982
Arctic char (*Salvelinas alpinus*)	5	NA	NA	NA	30.0	Skaftason and Johannesson, 1982
Atlantic salmon (*Salmo salar*)	6	NA	NA	NA	46.0	Skaftason and Johannesson, 1982

TABLE 6-10 (cont.)

		Mean Concentration (mg/kg) of Chlorinated Benzene				
Species/Tissue	Number Analyzed	Tri-	Tetra-	Penta-	Hexa-	Reference
Coho salmon (*Oncorhynchus kisutch*)						
Liver	28	NA	NA	NA	0.065[b]	Norstrom et al., 1978
Muscle	28	NA	NA	NA	0.097[b]	Norstrom et al., 1978
Brittle star (*Ophiura albida*)	15	NA	NA	1.10	21.2	Bjerk and Brevik, 1980
Hermit Crab (*Pagurus sp.*)	3	NA	NA	0.88	4.3	Bjerk and Brevik, 1980
Snail (*Littorina littorea*)	3	NA	NA	NA	13.9	Bjerk and Brevik, 1980
Sea star (*Asteroidea*)	12	NA	NA	0.78	1.03	Bjerk and Brevik, 1980
Saithe, homogenate (*Pollachius virens*)	13	NA	NA	1.11	21.8	Bjerk and Brevik, 1980

[a] Values are the concentration ranges for five sampling sites around Norway.

[b] Concentrations expressed as wet weight of fish.

Fish and invertebrates collected from contaminated waters have been shown to contain various levels of chlorinated benzenes. Only Ofstad et al. (1978) collected water and sediment samples for chlorinated benzene analysis and quantitatively confirmed the presence of tri- through hexachlorobenzenes in the area where contaminated fish were collected. Concentrations of chlorinated benzenes in the fish were inversely related to the distance of the collection site from a chlorinated benzene discharge point. Data from several reports of tissue levels of tri-, tetra-, penta- and hexachlorobenzene in fish from the United States, Canada and Norway are presented in Table 6-10. Brunn and Manz (1982) collected 72 samples of various fish species from several ponds, streams and rivers of Germany. Residues of hexachlorobenzene were present in 66 samples (92%) at average concentrations ranging from 0.265 mg/kg fat in fish from ponds without a flowing surface-water connection to 0.463 mg/kg fat in fish from rivers.

Additional data on tissue levels of chlorinated benzenes in fish and BCFs were discussed in Section 5.3.

6.2. EFFECTS ON NONAQUATIC ENVIRONMENTS

6.2.1. Plants.

Plant seedlings and germinating seeds are commonly exposed to 1,4-dichlorobenzene to disrupt or arrest mitosis and facilitate chromosome study (Meyer, 1948). Sharma and Bhattacharyya (1956) exposed healthy root tips of 10 monocotyledons and 6 dicotyledons to a saturated solution of 1,4-dichlorobenzene. Chromosome fragmentation was observed in all species after 1.5-4.5 hours of exposure. Barley, oat and wheat seedlings were raised in greenhouse pots of sand, sandy loam, clay loam or clay treated with 1,2,4,5-tetrachlorobenzene at application rates equivalent to 0, 1.9, 5.6, 16.9, 50.6 or 151.9 kg/ha (Ameen et al., 1960). Eighteen days after planting, a decrease was observed in seedling germination and in heights and root lengths of seedlings of all three varieties and in all four soil types. A gradient of severity was reported, however, decreasing from sand to sandy loam, clay loam and clay. No effects were noted in any variety grown in any soil type treated at the highest application rate (151.9 kg/ha) if planting was delayed 125 days. Mature cotton plants grown in Norfolk sandy loam soil were observed 30 days after soil treatment with 1,2,4,5-tetrachlorobenzene at application rates of 0-4483 kg/ha to control nematode parasites (Adams and Rodriquez-Kabana, 1976). There was 100% mortality in plots treated at ≥224 kg/ha. No effects on the cotton plants were observed at application rates of 0-112 kg/ha.

6.2.2. **Insects.** Pupae of the housefly, Musca vicina, were exposed to "saturation concentration" vapors of each of the three dichlorobenzene isomers for 3, 6 or 10 hours (Levinson, 1955). The emergence of adult flies 8 days after exposure is shown in Table 6-11. The actual concentrations of the various exposure atmospheres, however, were not reported.

Solutions of 1,2-dichlorobenzene in diesel oil (1:3 or 1:5 ratio dichlorobenzene:oil) and of an unspecified trichlorobenzene isomer in diesel oil (1:5 ratio) effectively eliminated all broods of the Douglas-fir beetle, Dendroctonus pseudotsugae, when sprayed on both fallen logs and standing trees (Gibson, 1957). The actual volumes of spray or total weight of the chlorobenzene applied were not specified.

Fifteen virgin female wasps, Bracon hebetor, were each placed overnight inside glass vials, the sides of which had been uniformly coated with a 10 ppm solution of 1,3,5-trichlorobenzene in 0.25 mℓ of acetone (Grosch and Hoffman, 1973). The mean lifespan of the females was shortened (15.7±1.1 days) compared with controls (22.0±0.8 days). Embryo mortality, measured by the number of unhatched eggs, in the control and treated groups was similar

TABLE 6-11

Emergence of Adult Houseflies 8 Days Following Exposure of Pupae to "Saturation Concentration" of Dichlorobenzene Vapors[a]

Chemical[b]	Emergence of Houseflies (%) Resulting from Exposure Period of: 3 hours	6 hours	10 hours
1,2-Dichlorobenzene	46±10[c]	15±6	0
1,3-Dichlorobenzene	15±5	0	0
1,4-Dichlorobenzene	2±2	0	0

[a] Source: Levinson, 1955

[b] Percent emergence of controls = 94±2%

[c] In an average of 10% of the cases recorded as unhatched pupae, the flies had died after having pushed through the pupal skin with their ptilinum, but with their thorax and abdomen inside the pupal skin.

for the first 5 days after treatment, but by the seventh day, 71% of the eggs from treated females were unhatched.

6.2.3. Birds. The toxicity of hexachlorobenzene was tested in Japanese quail (Coturnix coturnix japonica) by dietary administration at 0, 1, 5, 20 or 80 mg/kg diet for 90 days (Vos et al., 1971). A NOEL was reported at 1 mg/kg. At higher concentrations liver damage and porphyrin excretion increased in a dose-related fashion. At 80 mg/kg 5 of 15 quail died during the exposure period. There was a dose-related decrease in the hatchability of eggs, especially in groups treated at 20 and 80 mg/kg (Vos et al., 1971). Carpenter et al. (1983) also reported hepatic toxicity and porphyria in quail treated orally with 500 mg/kg/day hexachlorobenzene for 1, 2, 5 or 10 days. Most treatment-related changes occurred after the first dose of hexachlorobenzene.

Studies on the effects of chlorinated benzenes, predominantly hexachlorobenzene, on wild birds have primarily focused on the accumulation of contaminants in eggs and their effects on embryo survival and reproductive parameters. Gilbertson and Fox (1977) determined hexachlorobenzene levels in eggs of Herring Gulls, Larus argentatus, from Lake Erie, Lake Ontario and in northern Alberta (used as an "uncontaminated" control). Hexachlorobenzene residue levels in eggs were 1.37, 4.30 and 0.21 mg/kg (dry matter basis), respectively. There was a relationship between the number of embryos that developed to pipping stages and the final percent hatching, and the area from which they were collected. Of the eggs collected in "uncontaminated" areas (n=14), 85% developed to pipping and 69% hatched. Of those collected at Lake Erie (n=25), 83 and 53%, respectively, pipped and hatched. Lake Ontario-collected eggs (n=47) showed a significant ($p<0.05$) decrease in survival to pipping (39%) and hatchability (26%). Liver weights and porphyrin levels in embryos from Lake Ontario and Lake Erie were greater than those of the control group. Gilman et al. (1977) reported that hatching success for Herring Gull eggs from Lakes Superior, Huron, Erie and Ontario were 80, 72, 63 and 19%, respectively, which supports the data of Gilbertson and Fox (1977). Additional data on residue levels of chlorinated benzenes in eggs and wild birds will be discussed in Section 6.2.4.

6.2.4. Residues. Harp seals, Phagophilus groenlandicus, having a high percentage of body fat, were found to contain hexachlorobenzene residues (Rosewell et al., 1979). Forty of 42 seal pups contained hexachlorobenzene (concentrations unspecified), which was concluded to be transferred from adult to fetus and also through maternal nursing of the pups.

Subcutaneous adipose tissue from wild foxes, boars and deer (in Germany) was analyzed for hexachlorobenzene content (Koss and Manz, 1976). The average tissue levels (ranges) were 0.29 (0.02-0.77) mg/kg in 21 foxes, 0.71 (0.05-3.11) mg/kg in 7 wild boars and 0.03 (0.00-0.05) mg/kg in 6 female deer. The detection of 1,4-di-, 1,2,4-tri-, 1,2,3,4-tetra-, 1,2,4,5-tetra-, penta- and hexachlorobenzene (concentrations not reported) in samples of pooled body lipid from Lake Ontario Herring Gulls (L. argentatus) was reported by Hallett et al. (1982). Similarly, Szaro et al. (1979) reported that 8 of 28 Great Black-Backed Gulls, collected in Maine, had average tissue levels of 0.03 mg hexachlorobenzene/kg (wet weight). Ohlendorf et al. (1981) reported hexachlorobenzene residues at an average concentration of 0.23 mg/kg (wet weight) among 12 of 105 herons, including great blue herons, Ardea herodias. During the period 1971-1974, Barbehenn and Reichel (1981) examined 101 bald eagles, Haliaetus leucocephalus, and found 19 carcasses to contain an average concentration of 8.0 mg hexachlorobenzene/kg (lipid basis; 2.2% body weight as lipid). Kaiser et al. (1980) reported that 23 of 168 bald eagles collected during 1975-1977 had mean carcass levels of 0.08 mg hexachlorobenzene/kg wet weight. An Osprey, *Pandion haliaetus* (0.2 mg/kg), a great horned owl, *Bubo virginianus* (0.7 mg/kg), Swainson's hawk (up to 5.2 mg/kg) and starlings, *Sturnus vulgaris* (0.21 mg/kg) also were found to contain hexachlorobenzene residues (Wiemeyer et al., 1980; Blus et al., 1983; Bechard, 1981; White, 1979).

Reports on the residue levels of some chlorinated benzenes in bird eggs are summarized in Table 6-12. Hexachlorobenzene has been the most prevalent and persistent chlorinated benzene identified.

6.3. SUMMARY

As demonstrated in acute toxicity bioassays, the LC_{50} in fish generally decreases as the number of substituent chlorine atoms on the molecule increases (isomers vary). Chlorinated benzenes have adverse effects on the reproduction of invertebrates and fish. Monochlorobenzene tested in goldfish and largemouth bass, 1,3,5-trichlorobenzene tested in brine shrimp and the exposure of sheepshead minnows to 1,2,4,5-tetrachlorobenzene resulted in decreased hatching of eggs or embryo lethality and decreased survival of juvenile fish.

Adverse effects of chlorinated benzenes were also apparent in terrestrial organisms. Mitosis in seeds and seedlings was disrupted by 1,4-dichlorobenzene; 1,2,4,5-tetrachlorobenzene affected seed germination and seedling growth depending on soil type. Soil application rates of 224 kg/ha or higher of 1,2,4,5-tetrachlorobenzene were found to be phytotoxic to mature cotton plants. Dichlorobenzene vapors at "saturation concentrations" inhibited the emergence of housefly pupae, while 1,2-dichlorobenzene and trichlorobenzene each in diesel oil were toxic to Douglas-fir beetles.

TABLE 6-12

Chlorinated Benzene Residues in Bird Eggs

Compound	Species	Number Analyzed	Mean Concentration (mg/kg)	Location	Reference
Tetrachlorobenzenes	Herring Gull (*Larus argentatus*)	65	0.026	Lake Ontario^{a}	Hallett et al., 1982
		10	0.015	Lake Ontario^{b}	Hallett et al., 1982
		13	0.024	Lake Erie^{b}	Hallett et al., 1982
Pentachlorobenzene	Herring Gull (*Larus argentatus*)	65	0.039	Lake Ontario^{a}	Hallett et al., 1982
		20	0.024	Lake Ontario^{b}	Hallett et al., 1982
		20	0.025	Lake Erie^{b}	Hallett et al., 1982
		13	0.025	Lake Huron^{b}	Hallett et al., 1982
		20	0.022	Lake Superior^{b}	Hallett et al., 1982
		20	0.021	Lake Michigan^{b}	Hallett et al., 1982
Hexachlorobenzene	Herring Gull (*Larus argentatus*)	65	0.451	Lake Ontario^{a}	Hallett et al., 1982
		20	0.315	Lake Ontario^{b}	Hallett et al., 1982
		20	0.09	Lake Erie^{b}	Hallett et al., 1982
		20	0.115	Lake Huron^{b}	Hallett et al., 1982
		20	0.115	Lake Superior^{b}	Hallett et al., 1982
		20	0.12	Lake Michigan^{b}	Hallett et al., 1982
	Great Black-Backed Gull	28	0.03	Maine	Szaro et al., 1979
	Common tern (*Sterna hirundo*)	13	7.67^{c}	Lake Ontario	Gilbertson and Reynolds, 1972
	Double-Crested Cormorant (*Phalacrocorax auritus*)	9-10	0.016	Bay of Fundy	Zitko, 1976

TABLE 6-12 (cont.)

Compound	Species	Number Analyzed	Mean Concentration (mg/kg)	Location	Reference
Hexachlorobenzene (cont.)	Canvasback Duck (*Aythya valisineria*)	11	0.02	Nevada	Stendell et al., 1977
		51	0.01	Manitoba	Stendell et al., 1977
	Red-Breasted Merganser (*Mergus serrator*)	114	0.06	Lake Michigan	Haseltine et al., 1981
		92	0.05	Lake Michigan	Haseltine et al., 1981
	Common Merganser (*Mergus merganser*)	2	0.05	Lake Michigan	Haseltine et al., 1981
	Brown Pelican (*Pelecanus occidentalis*)	115	0.03	South Carolina	Blus et al., 1979
	Great Horned Owl (*Bubo virginianus*)	4	0.2	Ohio	Springer, 1980

[a] Data collected in 1977

[b] Data collected in 1978

[c] Based on dry weight of egg

Contact with residues of 1,3,5-trichlorobenzene shortened the lifespan of female wasps, and their eggs suffered high mortality within 7 days of exposure.

Although effects (mortality, decreased reproduction) of chlorinated benzenes on natural populations have not been adequately studied, tissue concentrations of several isomers were measured in a number of different species. Aquatic organisms (fish and invertebrates) and terrestrial species, alike, have been found to contain chlorinated benzenes. Tissue concentrations of the quantitated chlorinated benzenes were highest for hexachlorobenzene. The detection in North America and Europe of hexachlorobenzene in the eggs of birds and subcutaneous fat of wild animals suggests its widespread distribution in the environment.

7. MONOCHLOROBENZENE

Between 88.7 and 128.7 million kilograms of monochlorobenzene is estimated to be produced in the United States in 1983 (U.S. EPA, 1983). Monochlorobenzene is used primarily as an intermediate in the synthesis of organic chemicals and as a solvent in herbicides and paints (Hawley, 1977). It has been detected in samples of urban, ambient and indoor air, as well as in surface, drinking and industrial wastewater, and in water and sediments in a stream draining an abandoned waste site (see Section 4.3.). Residues of monochlorobenzene have been found in human adipose tissue, and studies indicate that it bioaccumulates in fish and other aquatic organisms (see Sections 5.3. and 5.4.). In addition to the exposure of workers involved in organic chemical synthesis, humans are likely to be exposed to monochlorobenzene via inhalation of air and ingestion of water.

7.1. PHARMACOKINETICS

7.1.1. **Absorption.** Quantitative studies on the absorption of monochlorobenzene are lacking. Toxic effects reported in humans after ingestion or inhalation indicate that monochlorobenzene is absorbed via these routes (Reich, 1934; Rosenbaum et al., 1947; Tarkhova, 1965). Studies of the metabolism of monochlorobenzene in a number of mammalian species indicate that absorption from the gastrointestinal tract does occur (Williams, 1959). Given the lipophilic character of monochlorobenzene and the dermal absorption of other chlorobenzenes, some degree of absorption through the skin would be expected.

7.1.2. **Distribution.** The only available study regarding the distribution of monochlorobenzene is the inhalation pharmacokinetic experiments of Sullivan et al. (1983). Male Sprague-Dawley rats were exposed to 100, 400 or 700 ppm of ^{14}C-monochlorobenzenene, 8 hours/day for 1 or 5 consecutive days. Following a single exposure period, radioactivity was found in all tissues examined both immediately and 48 hours post-exposure, with the highest concentrations located in the fat. With the exception of the kidney, five exposures did not result in significantly higher tissue concentrations than did a single exposure, indicating that a steady-state concentration is reached during the first 8 hours of exposure. The tissue concentration was proportional to the exposure concentration, with the exception of the fat, in which the tissue levels increased 8- to 10-fold when the exposure concentration was increased from 100-400 ppm and 3- to 5-fold when the exposure concentration was increased from 400-700 ppm.

7.1.3. Metabolism.

Shimada (1981) administered monochlorobenzene to rats (strain and dose unspecified) by subcutaneous injection and analyzed the urine by high performance liquid chromatography. They detected p-chlorophenylmercapturic acid and monoglucuronide and ethereal sulfate conjugates of 4-chlorocatechol. Based on this information, they proposed that the metabolism of monochlorobenzene involves an initial oxidation to form 4-chlorobenzene-1,2-epoxide. This intermediate may then 1) form a glutathione conjugate, resulting in the excretion of p-chlorophenylmercapturic acid, 2) be converted to 4-chlorophenol, conjugated, and excreted, or 3) be converted to 4-chlorocatechol, conjugated, and excreted.

Nakajima and Sato (1979) found that fasting enhanced the activity of liver enzymes for monochlorobenzene in both male and female Wistar rats. They found that fed male rats metabolized most of the hydrocarbons tested more rapidly than fed female rats; however, there were no significant differences in the initial metabolic rate for monchlorobenzene between sexes.

Selander et al. (1975) investigated the metabolism of monochlorobenzene in perfused rat livers and in a variety of cell-free hepatic preparations

(Table 7-1). They found that monochlorobenzene is converted to chlorophenols by three different enzymes. Two of these enzymes form arene oxide intermediates (3- and 4-chlorobenzene oxides) resulting in the formation of *o*- and *p*-chlorophenol. *m*-Chlorophenol appeared to occur via a direct oxidative pathway. Under the conditions of these assays, conjugation of the arene oxide with glutathione or hydration did not occur to a significant extent.

Smith et al. (1972) administered 75 μCi (0.59 gm) of ^{14}C-monochlorobenzene emulsified in Cremophor E.L. and physiological saline to two ~1.5 kg female Dutch rabbits by gavage, twice a day for 4 days. The major urinary metabolites recovered were *p*-chlorophenylmercapturic acid and the conjugates of 4-chlorocatechol. Other minor metabolites detected were quinol, 3-chlorocatechol, *o*-chlorophenylmercapturic acid and *m*-chlorophenylmercapturic acid. The identified metabolites accounted for over 98% of the urinary radioactivity and consisted of 3,4-dihydro-3,4-dihydroxychlorobenzene (0.57%), monophenols (2.84%), diphenols (4.17%), mercapturic acids (23.80%), ethereal sulfates (33.88%) and glucuronides (33.57%).

7.1.3.1. **TISSUE BINDING** -- Reid (1973) and Reid et al. (1973) have studied tissue distribution and tissue binding of monochlorobenzene and the related halobenzene, bromobenzene. Treatment of C57B6J mice with a single intraperitoneal dose of 4.58 mmol/kg bromobenzene or 6.75 mmol/kg monochlorobenzene produced necrosis of the proximal convoluted tubules of the kidneys within 48 hours. This was associated with covalent binding of an unidentified ^{14}C labeled metabolite to the site of necrosis prior to manifestation of the histologic effect. Pretreatment of animals with pyrazole butoxide blocked the binding as well as the toxic effect. Six hours after administration of 1 mmol/kg (112 mg/kg), ~0.332 nmol equivalents of ^{14}C-monochlorobenzene/mg protein were covalently bound. Although

TABLE 7-1

Percentage of Isomers of Chlorophenol from Metabolism of Monochlorobenzene*

System	Isomer (%) ortho-	meta-	para-
Perfused liver	40	20	40
Phenobarbital treated	46	10	44
Methylcholanthrene treated	89	2	9
Microsomes	18	7	75
Phenobarbital treated	32	6	62
Methylcholanthrene treated	59	6	35

*Source: Selander et al., 1975

tissue distribution was not studied with monochlorobenzene, a metabolite of bromobenzene was strongly bound by tissues from the liver, lungs and kidneys but not by tissue from the heart, spleen or testes, and this binding correlated with necrotic changes. Microsomes from the lungs and liver (*in vitro*) oxidized bromobenzene, whereas microsomes from the kidneys, heart, spleen and testes did not, which indicated that metabolic activation took place in lungs and liver and that an active metabolite was transported to the kidneys before binding. Pretreatment with 3-methylcholanthrene enhances the overall metabolism of monochlorobenzene; however, this pretreatment reduces the extent of covalent binding to cellular macromolecules and prevents centrolobular hepatic necrosis (Reid et al., 1971). Similar results of preventing chlorobenzene-elicited liver necrosis have been obtained by inhibiting epoxide hydrase with cyclohexene oxide (Oesch et al., 1973). Jergil et al. (1982) found that when monochlorobenzene was incubated with liver microsomes it was bound to microsomal proteins of molecular weights of 72,000 and 50,000-60,000 daltons. The metabolite probably was bound to the sulfhydryl groups of proteins, since the addition of glutathione blocked the binding.

7.1.4. **Excretion.** Sullivan et al. (1983) exposed male Sprague-Dawley rats to atmospheres containing ^{14}C-monochlorobenzene (100, 400 or 700 ppm) 8 hours/day for 1 or 5 days. Following treatment, the label was detected in the expired air and urine of the rats. The urine contained metabolites of monochlorbenzene, including mercapturic acids, glucuronide conjugates and sulfate conjugate; the respiratory elimination consisted of unmetabolized compound. The percentage of the dose excreted by respiration increased with increasing exposure, implying that the metabolic elimination of monochlorobenzene can be saturated.

Smith et al. (1972) orally dosed two female Dutch rabbits with 0.5 g (75 μCi) of ^{14}C-monochlorobenzene emulsified in Cremophor E.L. and physiological saline twice a day for 4 days, and collected urine and feces throughout the 7 days of the study. The urine contained 19.6% of the administered label, the feces (methanol extracted) contained 1.05% and the tissues contained 0.05%. Radiolabeled ^{14}C in expired air was not measured. Williams (1959) has reported that 27% of a 0.5 g/kg dose orally administered to rabbits was excreted in expired air over a 1-2 day period.

Lindsay-Smith et al. (1972) found that the conjugated metabolites were both mono- and diphenolic in the rabbit, but the monophenolics were predominate. p-Monochlorophenol was the predominant isomer in the urine. The distribution of isomers for free and conjugated monochlorophenol combined was: ortho-, 4.9%; meta-, 22.9%; and para-, 72.2%. For free monochlorophenols, the distribution of isomers was 5.9, 33.6 and 60.4% for ortho-, meta- and para-isomers, respectively. The major diphenolic metabolite was reported to be 4-chlorocatechol; small amounts of chloroquinol, 3-chlorocatechol and quinol also were found. Although there was not adequate proof in these studies, it was proposed that metabolism proceed through the formation of an arene oxide (3,4-chlorobenzene oxide). Conjugation of this arene oxide with glutathione followed by further metabolic reactions would account for the meta- and para-chlorophenyl mercapturic acids but not the ortho-isomer. Hydration of the arene oxide, followed by dehydrogenation, would lead to chlorocatechol. Pathways for metabolism have been proposed based on the *in vivo* and *in vitro* studies (Figure 7-1).

The profile of urinary metabolites varies from species to species. For example, Williams et al. (1975) reported on 13 species and indicated that 19-65% of ^{14}C-urinary metabolites of monochlorobenzene was p-chloro-

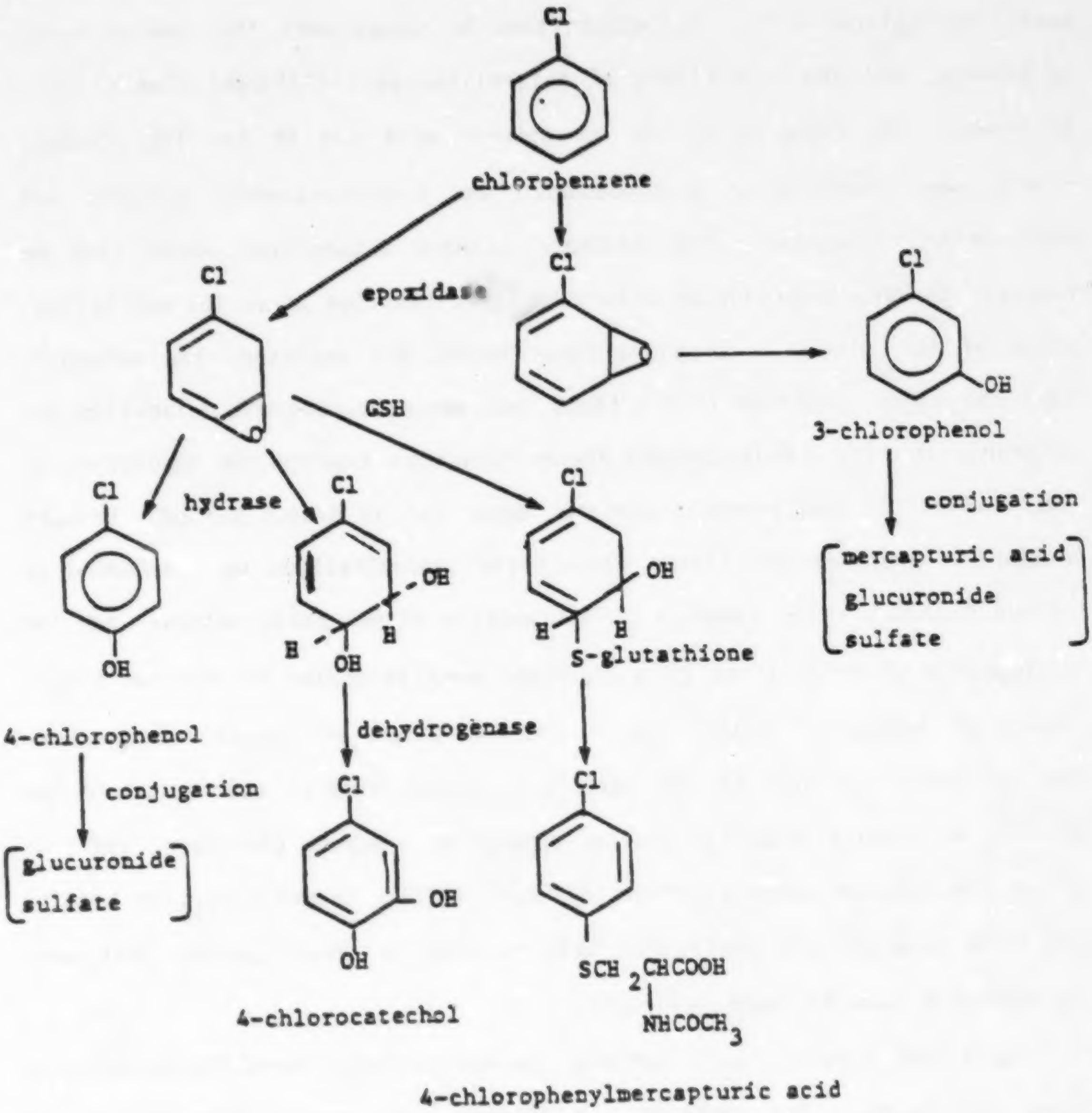
Cl
chlorobenzene
epoxidase
Cl
Cl
GSH
3-chlorophenol
conjugation
mercapturic acid
glucuronide
sulfate
Cl
hydrase
Cl
Cl
OH
OH
H
H
OH
H
H
S-glutathione
OH
4-chlorophenol
dehydrogenase
conjugation
glucuronide
sulfate
Cl
Cl
OH
OH
SCH 2 CHCOOH
NHCOCH3
4-chlorocatechol
4-chlorophenylmercapturic acid

FIGURE 7-1

Metabolism of Monochlorobenzene

Adapted from: Williams, 1959; Lindsay-Smith et al., 1972; Selander et al., 1975; Shimada, 1981; Sullivan, 1981 phenyl mercapturic acid. The metabolites in humans were the same as those in animals, but the proportions of metabolites were different (Table 7-2). In humans, 19% appeared as the mercapturic acid and 33 and 31%, respectively, were excreted as 4-chlorophenol and 4-chlorocatechol sulfate, and glucuronide conjugates. The ultimate urinary metabolites would also be expected to vary depending on saturation of metabolism or on the nutritional state of the animals. If glutathione levels are depleted, the metabolic fate can vary. Sullivan (1981) found that monochlorobenzene metabolism was saturable in rats. Male Sprague-Dawley rats were exposed via inhalation to 100, 400 or 700 ppm monochlorobenzene vapor for an 8-hour period. Urinary metabolite profiles and tissue glutathione concentrations were measured at 16 and 48 hours after exposure. The capacity of metabolic oxidases and the conjugation of metabolites to glutathione were saturated at the two higher levels of exposure. Saturation of detoxification mechanisms can increase the incidence and severity of toxicity. Recent studies have examined the profile of urinary metabolites after inhalation exposure (Sullivan, 1981) or after subcutaneous administration (Shimada, 1981). Essentially, the results of these studies are consistent with results in other species that were administered monochlorobenzene orally.

Ogata and Shimada (1982) compared the metabolism of monochlorobenzene in rats and humans. The compound was diluted with polyethylene glycol and injected intraperitoneally into rats or administered to rats and human volunteers orally. Urine specimens were also collected from two workers at a factory involved in distilling monochlorobenzene. In rats, the major metabolite detected was p-chlorophenylmercapturic acid, accounting for 6-10 times the amount of material excreted as conjugates of 4-chlorocatechol. In a human volunteer, only trace amounts of p-chlorophenylmercapturic

TABLE 7-2

Species Variation in Urinary Metabolites of ^{14}C-Monochlorobenzene*

Species	Percentage of 24-Hour Excretion of ^{14}C		
	4-Chlorophenol	4-Chlorocatechol	4-Chlorophenyl-Mercapturic Acid
Man	33	31	19
Rhesus monkey	19	37	40
Squirrel monkey	14	37	50
Capuchin monkey	19	36	41
Dog	14	45	42
Ferret	33	31	24
Hedgehog	20	12	65
Rabbit	29	38	26
Rat	23	22	49
Mouse	20	31	42
Gerbil	13	26	51
Hamster	15	23	43
Guinea pig	27	35	21

*Source: Data cited by Williams et al., 1975 acid were detected; however, conjugates of 4-chlorophenylmercapturic acid were the major metabolites observed. These findings suggest that urinary 4-chlorocatechol conjugates may be used to monitor human exposure to monochlorobenzene.

7.1.5. **Summary.** Monochlorobenzene is readily absorbed by inhalation and by the gastrointestinal tract but the quantitative extent is not known. It is deposited in body lipids and metabolized by microsomal oxidation. Oxidative reactions lead to the formation of arene oxides; these epoxides are metabolized further to the ortho-, meta- or para-chlorophenols. The chlorophenols may conjugate with glutathione and be detoxified by conversion to the corresponding mercapturic acids and excreted in the urine or they may bind to cellular proteins. Binding to cellular protein appears to be correlated with necrotic pathologic changes in the kidneys and livers of rodents. In addition to conjugation with glutathione, metabolites of monochlorobenzene (monophenols and diphenols) can conjugate with glucuronic acid or with sulfate and be excreted in the urine. Monophenols are the predominant metabolites; the diphenols are minor. The arene oxides, 3-chlorobenzene oxide or 4-chlorobenzene oxide, also can be converted to the dihydrodiol by epoxide hydrase and dehydrogenated to form chlorocatechols. There appear to be species differences in the profile of urinary conjugation of metabolites, and end metabolites may vary depending on the availability of tissue glutathione. Detoxification by conjugation with glutathione is important in the modulation of toxic effects especially at high exposure levels.

7.2. EFFECTS ON HUMANS

No epidemiologic studies regarding the effects of exposure to monochlorobenzene are available. Several case studies and one clinical study, however, provide some information regarding the toxic effects of the chemical in humans. Maximum allowable air concentrations range from 75 ppm in the United States and Switzerland to 11 ppm in Sweden (Merian, 1980).

Reich (1934) reported the case of a 2-year-old boy who swallowed ~5-10 mℓ of monochlorobenzene. Within 2 hours, the child's lips were cyanotic, and he had no detectable reflexes. He became unconscious and cyanotic and displayed head and neck twitching. He regained consciousness after ~3 hours and all signs returned to normal within 8 hours. There was no followup on the patient.

Girard et al. (1969) reported the case of a 70-year-old woman who had worked for 6 years with a glue containing 70% monochlorobenzene. From the time when she began using the glue, her symptoms included headaches and irritation of the upper respiratory tract and the eye mucosa. After 6 years of exposure hematologic examination resulted in a diagnosis of medullar aplasia.

Rosenbaum et al. (1947) examined 28 factory workers who had been exposed to monochlorobenzene for 1-2 years. Many of the workers complained of headaches and showed signs of somnolescence and dyspepsia. Eight of the 28 had tingling, numbness and stiffness of the extremities, eight had hyperesthesia of the hands, nine had spastic contractions of the finger muscles, and two had spastic contractions of the gastronemius muscle. Twenty-six workers who had either short-term exposure (<1 year) to monochlorobenzene or exposure to combinations of benzene and monochlorobenzene fumes displayed no neurotoxic signs.

Tarkhova (1965) exposed 4 humans to 0.02, 0.04 or 0.06 ppm (0.1, 0.2 or 0.3 mg/m^3) of monochlorobenzene and monitored electroencephalographic patterns. At the lowest concentration there were no effects, but within minutes at the higher doses, there were changes in response patterns to 10-nanosecond light flashes of 8-10 Hz.

Human exposure to monochlorobenzene by inhalation or by accidental ingestion can cause neurotoxic signs (Reich, 1934; Rosenbaum, 1947). It is not known if the effects are reversible after long-term exposure or if there are other sites of toxicity.

7.3. MAMMALIAN TOXICITY

7.3.1. Acute Toxicity. Treatment with monochlorobenzene has been demonstrated to produce a variety of changes in enzymatic and physiological function, including the slight depression of mitochondrial oxidative phosphorylation in male Donryu rats (Ogata et al., 1981), increased flow of bile duct-pancreatic fluid in male Holtzman rats (Yang et al., 1979), stimulating the activity of δ-aminolevulinic acid synthetase and hemeoxidase in male Wistar rats (Ariyoshi et al., 1981) and decreasing hepatic cytochrome P-450 in female Wistar rats (Ariyoshi et al., 1975).

Varshavskaya (1967) investigated the toxicological, olfactory and gustatory properties of monochlorobenzene and ortho- and para-dichlorobenzene. The olfactory and gustatory thresholds were found to be 0.01-0.02 mg/ℓ for monochlorobenzene. In the oral toxicity tests with albino rats, the highest concentration of monochlorobenzene that produced no observed toxic effect was 0.001 mg/kg.

Rimington and Ziegler (1963) administered monochlorobenzene to male albino rats by daily gastric intubation, using an escalating dosage regimen. Monochlorobenzene was less effective in producing porphyria than were 1,4-dichlorobenzene, 1,2,4-trichlorobenzene or 1,2,3,4-tetrachlorobenzene (hexachlorobenzene was not studied). Monochlorobenzene also has been observed to produce bronchiolar necrosis (Reid et al., 1973) and centrolobular hepatic necrosis (Reid and Krishna, 1973).

A summary of the acute lethal doses of monochlorobenzene is presented in Table 7-3. In a majority of the studies reviewed, fatalities were the result of central nervous system depression. Irish (1963) reported that cats tolerated monochlorobenzene at concentrations of 220-660 ppm for 1 hour. Narcotic signs were noted at levels of 1200 ppm, and death occurred after 7 hours of exposure at 3700 ppm. Cats exposed at 8000 ppm for 30 minutes died 2 hours after exposure. By the oral route, LD_{50} values in rats and rabbits were reported to be 2.91 and 2.83 g/kg bw, respectively. Bonnet et al. (1982) reported that 6-hour inhalation exposures to rats and mice resulted in LC_{50}s of 2965 and 1886 ppm, respectively.

Administration of sublethal doses of monochlorobenzene causes toxic signs that are manifest within 24 hours. When mice are given a single intraperitoneal dose of 6.75 mmol/kg (760 mg/kg), they develop coagulation necrosis of the proximal tubules of the kidneys. Rats are slightly less sensitive. Doses of 9.3 mmol/kg (1047 mg/kg) have been reported to cause swollen, vacuolated, convoluted tubules (Reid et al., 1971).

Monochlorobenzene causes sensory irritation of the respiratory system. A comparison of the index of sensory irritation for 22 chemicals was made based on a short inhalation experiment in mice (De Ceaurriz et al., 1981). Mice were exposed usually for 5 minutes at varying concentrations, and respiratory rates were measured with a plethysmograph. An RD_{50} value for mice, which is the concentration that causes a 50% decrease in respiratory rate, was calculated. An uncomfortable human dose was predicted to be 0.1 RD_{50}, and a no-effect dose was predicted to be 0.01 RD_{50}. For monochlorobenzene, the RD_{50} was 1054 ppm, and the predicted no-effect human dose was 11 ppm. For comparison, the RD_{50s} for formaldehyde and toluene diisocyanate were 5.3 and 0.24 ppm, respectively.

TABLE 7-3

Acute Toxicity of Monochlorobenzene

Species	Route	Dose	Exposure Duration (hour)	Lethal Effect Level	Reference
Rat	inhalation	22,000 ppm 9,000 ppm	2.5 3.0	LC_{50} LC_{67}	Eastman Kodak, 1978
Cat	inhalation	3,700 ppm 8,000 ppm	7.0 0.5	LC_{100} LC_{100}	Irish, 1963
Rat, Sprague-Dawley	inhalation	2,965 (2787-3169)* mg/kg	6.0	LC_{50}	Bonnet et al., 1982
Mouse	inhalation	1,886 (1781-1980)* mg/kg	6.0	LC_{50}	Bonnet et al., 1982
Rat	oral	2,144 mg/kg	NR	LD_{50}	Monsanto, 1965
Rat	oral	400-1600 mg/kg	NR	LD_{50}	Eastman Kodak, 1978
Rabbit	oral	2,830 mg/kg	NR	LD_{50}	Eastman Kodak, 1978
Rat	i.p.	7,400 mg/kg	NR	LD_{50}	NIOSH, 1982
Guinea pig	i.p.	4,100 mg/kg	NR	LD_{50}	NIOSH, 1982
Rabbit	dermal	>10 g/kg	NR	LD_{50}	Monsanto, 1965

*95% confidence limits in parentheses

NR = Not reported

Biochemical manifestations of the acute toxic effects of monochlorobenzene may be associated with the binding of liver and kidney protein by metabolites of the compound (the arene oxide or monochlorophenol) as discussed in Section 7.1.2. Ogata et al. (1981) found that 0.24 mM monochlorobenzene, in an _in vitro_ assay, caused a slight depression of rat liver mitochondrial oxidative phosphorylation. This effect was much less than the effect caused by more highly chlorinated congeners and may be correlated with a slight decrease in cytochrome P-450 in the liver of rats administered 200 mg/kg monochlorobenzene intraperitoneally 24 hours before analysis (Ariyoshi et al., 1975).

7.3.2. Subchronic Toxicity. The subchronic toxicity data are summarized in Table 7-4. Several investigators have studied the subchronic inhalation toxicity of monochlorobenzene. Dilley (1977) exposed groups of 32 male Sprague-Dawley rats (125 g) or male rabbits (2.0-2.5 kg) to monochlorobenzene (99+%) at 0, 75 and 250 ppm for 7 hours/day, 5 days/week, for 24 weeks. After exposure for 11 weeks (55 exposures), the rats showed increased liver-to-body weight ratios. After 120 exposures at 250 ppm, the rats showed an increase in liver-to-body and kidney-to-body weight ratios as well as decreased food consumption. Slight changes were also observed in three hematologic parameters (reticulocyte, white blood cell, and platelet counts). Histopathologic changes were seen in the kidneys, liver and adrenals of rats at 11 and 24 weeks; the kidneys had regenerating cortical tubules with basophilic inclusions in the cytoplasm of cells, the livers were congested and the adrenals had vacuolation of cells in the zona fasciculata. It was suggested that 75 ppm may be the marginal toxic concentration for daily inhalation. Effects were less marked in rabbits than in rats; no histologic or hematologic changes were found relating to monochlorobenzene exposures at 24 weeks.

TABLE 7-4

Summary of Subchronic Toxicity Studies on Monochlorobenzene[a]

Species	Route	Dose	Duration (days)	Effects	Reference
Dog (beagle)	inhalation[b]	0.75 mg/ℓ, 6 hrs/day, 5 days/week (162 ppm)	62 exposures over 90 days	None	Monsanto, 1978
		1.50 mg/ℓ, 6 hrs/day, 5 days/week (424 ppm)	62 exposures over 90 days	Weight loss; conjunctivitis; moribund at 31 days	
		2.00 mg/ℓ, 6 hrs/day, 5 days/week	62 exposures over 90 days	Weight loss; hypoactivity and conjunctivitis; vacuolated hepatocytes; cytoplasmic vacuolation of renal collecting tubules; bilateral atrophy of seminiferous tubules; lower total leukocyte counts, elevated SAP, SGOT, SGPT; aplastic bone marrow; mortality in 5/8 dogs after 25-29 days	
Rat	inhalation	0.75, 1.50 or 2 mg/ℓ 6 hrs/day, 5 days/week	62 exposures over 90 days	None	Monsanto, 1978
Rat	inhalation	0.1 or 1.0 mg/m^3 (continuous)	72-80	Liver necrosis and regeneration; kidney hyperplasia; encephalopathy; pneumonia	Khanin, 1977
Rat	inhalation	0.1 mg/m^3 (continuous)	60	None	Tarkhova, 1965
		1.0 mg/m^3 (continuous)	60	Inhibited chronaxia of antagonistic muscles at 39 days; increased blood cholinesterase	
Rat	inhalation	0.1, 1.25 or 1.5 mg/ℓ	49-98	Chronaximetric inhibition	Pislaru, 1960
Rat	inhalation	0.1 mg/ℓ, 3 hr/day (alternate days)	37 weeks	Inhibition of extensor tibialis 7-14 weeks; normal by 20 weeks	Gabor and Raucher, 1960
Rat	inhalation	75 and 250 ppm, 7 hrs/day 5 days/week	120 exposures	Focal lesions of adrenal cortex; lesions in tubules of kidneys; congestion of liver and kidneys; decreased SGOT	Dilley, 1977
Rabbit	inhalation	75 and 250 ppm, 7 hrs/day, 5 days/week	120 exposures	Decreased SGOT after 24 weeks of exposure	Dilley, 1977

TABLE 7-4 (cont.)

Species	Route	Dose	Duration (days)	Effects	Reference
Mouse	oral (gavage)	60 mg/kg/day, 5 days/week	13 weeks	one male with hepatic necrosis	NTP, 1983
		125 mg/kg/day, 5 days/week	13 weeks	Increased liver weights in males one male with hepatic necrosis	
		250 mg/kg/day, 5 days/week	13 weeks	>50% reduction in weight gain, increased excretion of coproporphyrins in females, increased liver weights, lesions of the liver, kidney, bone marrow, spleen and thymus	
		500 mg/kg day, 5 days/week	13 weeks	100% lethal to males within 1 week, reduced body weight gains, polyuria in females, increased liver weights, lesions of the liver, kidney, bone marrow, spleen and thymus.	
		750 mg/kg/day, 5 days/week	10 weeks	100% lethal to male mice within 1 week and to female mice within 10 weeks, lesions of the liver, kidney, bone marrow, spleen and thymus at death	
Rat	Oral (gavage)	60 mg/kg/day, 5 days/week	13 weeks	None	NTP, 1983
		125 mg/kg/day, 5 days/week	13 weeks	None	
		250 mg/kg/day, 5 days/week	13 weeks	Minimal centrolobular hepatocellular necrosis	
		500 mg/kg/day, 5 days/week	13 weeks	Decreased body weights gain, increased GGTP and alkaline phosphatase in females, increased excretion of porphyrins, controlobular hepatocellular necrosis, nephropathy in males, myeloid depletion of bone marrow.	
		750 mg/kg day, 5 days/week	13 weeks	Decreased body weight gain and survival of animals, hematologic effects, increased GGTP and alkaline phosphatase in females, polyuria in males, increased excretion of porphyrins, centrolobular hepatocellular necrosis, nephropathy, lymphoid depletion of thymus and spleen, myeloid depletion of bone marrow.	

TABLE 7-4 (cont.)

Species	Route	Dose	Duration (days)	Effects	Reference
Dog	oral (capsule)	27.3 mg/kg/day	90	None	Monsanto, 1967a
		54.6 mg/kg/day	90	Diarrhea and vomiting; conjunctivitis	
		272.5 mg/kg/day	90	4/8 died in 3-5 weeks; increased immature leukocytes; elevated SGOT and SAP, bilirubin and cholesterol; low blood sugar; histopathologic changes in liver, kidneys, spleen	
Rat	oral (diet)	12.5 or 50 mg/kg/day	93-99	None	Monsanto, 1967b
		100 mg/kg/day	93-99	Increased liver and kidney weights	
		250 mg/kg/day	93-99	Increased liver and kidney weights; retarded growth in males	
Rat	oral (diet)	14.4 mg/kg/day	192	None	Irish, 1963
		144 and 288 mg/kg/day	192	Increased liver and kidney weights; increased salivation and hair loss	

[a] Source: Updated from U.S. EPA, 1980a

[b] 1 ppm ~4.60 mg/m^3, 1 mg/ℓ ~219 ppm (Irish, 1963)

Monsanto (1978b) exposed by inhalation Charles River albino rats to monochlorobenzene at 0, 0.76, 1.47 and 2.00 mg/ℓ (0, 165, 319 and 434 ppm), 6 hours/day, 5 days/week for 62 exposures. Fifteen rats of each sex were exposed at each dose level. Erythema and hair loss were noted in 2 of 30 animals at the lowest dose. Hematology, clinical chemistry values and urinalysis parameters were found to be similar between the treated and control groups, and no histopathologic changes attributable to monochlorobenzene were found.

Beagle dogs exposed under the same regimen as the rats had toxic manifestations. Although no effects were noted at 0.75 mg/ℓ, at 1.5 mg/ℓ, 2 of 8 dogs were moribund and sacrificed at 30 days; they were hypoactive, had decreased weight gain, and conjunctivitis. No clinical or histopathologic examination was made on this group. At the 2.0 mg/ℓ level, all the dogs displayed weight loss, hypoactivity, and conjunctivitis. The mean leukocyte counts of these dogs were lower than in controls at 45 and 90 days, and SAP and SGOT were elevated at 38 days. Five dogs were moribund and therefore sacrificed between days 25 and 38. Histopathologic examination revealed vacuolization of hepatocytes in 5 of 8 dogs, aplastic bone marrow in 5 of 8 dogs, abnormalities of collecting tubules of the kidneys in 4 of 8 dogs, and bilateral atrophy of seminiferous tubules in 2 of 4 dogs.

Tarkhova (1965) exposed by inhalation adult male rats to monochlorobenzene at 0.1 or 1.0 mg/m^3 (0.02 or 0.2 ppm) for 60 days of continual exposure. No effects were seen at the lower level, but neurotoxic effects were noted at the higher level. In the high dose group, the chronaxy ratio of antagonistic muscles was reversed at day 39 (i.e., the conduction speeds of nerve impulses to sets of flexor and extensor muscles had changed). Blood cholinesterase was increased before the chronaximetric changes developed. Similar neurotoxic effects in rats were reported by Pislaru (1960) and Gabor and Raucher (1960).

Subchronic toxicity studies regarding the effects of monochlorobenzene administered to rats and dogs via gavage (oral administration) have been reported by Monsanto (1967a,b). Male and female rats (18 of each sex in each group) were dosed with 0, 12.5, 50, 100 or 250 mg/kg monochlorobenzene in corn oil for 5 days/week for 13 weeks. There were no effects on mortality, no clinical signs of abnormality and no histopathologic lesions. A slight decrease in growth rate over controls in males receiving the highest dose level and a dose-related increase in salivation in the animals were noted.

Groups of 4 male and 4 female beagle dogs were given repeated doses of 27.3, 54.6 and 272.5 mg/kg of monochlorobenzene by capsule for 5 days/week for 13 weeks. At the highest dose, two animals died and two were moribund and sacrificed in the interval between 14 and 21 doses. All animals given doses of 272.5 mg/kg had weight loss and histologic changes in the liver, kidneys, gastrointestinal mucosa and hematopoietic tissues. Minimal histologic changes were seen at 54.6 mg/kg, and no effects were noted at the lowest dose. Animals that survived the higher dose had increases in SGPT, SAP, bilirubin and cholesterol.

Subchronic toxicity studies on monochlorobenzene were conducted under the auspices of the National Toxicology Program (NTP, 1983b). The investigations were completed using 10 male and 10 female $B6C3F_1$ mice and using 10 male and 10 female F344/N rats. The monochlorobenzene was administered by gavage using a corn oil vehicle, 5 mℓ/kg bw, 5 days/week for 13 weeks. The monochlorobenzene doses used were 0, 60, 125, 250, 500 and 750 mg/kg bw.

The mouse study resulted in 13-week survival rate of 100% (10/10), 100% (10/10), 100% (10/10), 44% (4/9), 0% (0/10) and 0% (0/10) in male mice and 90% (9/10), 100% (10/10), 100% (10/10), 60% (6/10), 30% (3/10) and 0% (0/10) in female mice for the 0, 60, 125, 250, 500 and 750 mg/kg dose groups, respectively (NTP, 1983b). Body weight gains during the 13 weeks were decreased when compared with control animals in the surviving male mice, 27% for the 60 and 125 mg/kg groups, and 82% for the 250 mg/kg group. A decrease in body weight gains in surviving female mice was seen only in the 250 and 500 mg/kg dose groups (50% decrease in both groups). No clear compound-related effects were found in the surviving monochlorobenzene-treated mice from the hematologic and clinical analyses performed. Polyuria was noted in the 750 mg/kg male group and the 500 mg/kg female group. Significantly increased excretion of coproporphyrins were observed in surviving female mice receiving 250 and 500 mg/kg. No changes in liver porphyrin concentrations were observed in any of the male or female mice. At sacrifice increased liver weights were observed in surviving male mice at 125 and 250 mg/kg and surviving female mice at 250 and 500 mg/kg. Dose dependent monochlorobenzene-induced injury was revealed after histologic examination of liver, kidney, bone marrow, spleen and thymus. Except for two male mice each with hepatic necrosis in the 60 and 125 mg/kg dose groups, the observed tissue injuries, which were graded as severe, only occurred in the 250, 500 and 750 mg/kg dose groups. The liver lesions consisted of focal hepatocytic necrosis and centrilobular hepatocyte degeneration at 250 mg/kg and centrilobular hepatocellular necrosis at 500 and 750 mg/kg dose levels. Nephropathy was observed in female mice at 250 mg/kg dose, and in male mice at 250, 500 and 750 mg/kg doses. Both sexes of mice had myeloid depletion of the bone marrow at doses $\geq$250 mg/kg. Doses of $\geq$250 mg/kg caused necrosis of the thymus and doses of ≥500 mg/kg caused lymphoid depletion in the thymus. Based on these results, 60 mg/kg should be considered a lowest-observed-adverse-effect level (LOAEL).

The rat study resulted in a 13-week survival rate of 90% (9/10), 100% (10/10), 100% (10/10), 100% (10/10), 60% (6/10) and 10% (1/10) in male rats and 100% (10/10), 100% (10/10), 100% (10/10), 100% (10/10), 70% (7/10) and 20% (2/10) in female rats for the 0, 60, 125, 250, 500 and 750 mg/kg dose groups, respectively (NTP, 1983b). Body weight gains over the 13-week period were depressed by 10% or more in the male rats receiving doses ≥250 mg/kg and in female rats receiving 500 and 750 mg/kg doses. The only hematologic effects noted were at the 750 mg/kg dose level in surviving males (increased reticulocyte percentage) and females (decreased white blood cell count). The only consistent effects observed in the serum chemistries were slightly increased activities of γ-glutamyl transpeptidase and alkaline phosphatase in female rats receiving 500 and 750 mg/kg. The 750 mg/kg male rats were observed to have a doubling of their 24-hour urine output. Increased urinary excretion of uroporphyrins was observed in male rats at 750 mg/kg dose and of coproporphyrins in male rats at 500 and 750 mg/kg doses and in female rats at 500 mg/kg dose. No changes were observed in hepatic porphyrin levels. At sacrifice, monochlorobenzene-related histological changes were found in the liver, kidney, bone marrow, spleen and thymus. Liver lesions were classified as centrilobular hepatocellular necrosis (minimal at 250 mg/kg, minimal to moderate at 500 mg/kg, and moderate at 750 mg/kg for both sexes of rats). Mild to moderate nephropathy was observed in male and female rats at 750 mg/kg and in male rats at 500 mg/kg. Both male and female rats exhibited lymphoid depletions of the thymus and spleen at the 750 mg/kg dose and myeloid depletion of the bone marrow at the 500 and 750 mg/kg doses. From these rat data the lowest-observed-adverse-effect level (LOAEL) is 250 mg/kg and the NOEL is 125 mg/kg.

7.3.3. Chronic Toxicity. Two-year chronic bioassay studies using monochlorobenzene were conducted under the auspices of the National Toxicology Program (NTP, 1983b). The investigations were conducted using 50 male and 50 female B6C3F$_1$ mice and 50 male and 50 female F344/N rats. Monochlorobenzene was administered by gavage in a corn oil vehicle, 5 mℓ/kg, 5 days/week for 103 weeks. The dosage groups used were untreated, 0, 60 and 120 mg/kg for male and female rats and female mice, and untreated, 0, 30 and 60 mg/kg for male mice.

The mouse study revealed no monochlorobenzene-related clinical signs of toxicity or differences in mean body weights among test groups during the 105-week test period (exposure duration 103 weeks). Survival rates over the test period in the male mice were 70% (35/50), 78% (39/50), 56% (28/50) and 58% (29/50) for the untreated control, vehicle control (0), 30 and 60 mg/kg dose groups, respectively. Survival rates for the female mice were 74% (37/50), 80% (40/50), 82% (41/50) and 76% (38/50) for the untreated controls, vehicle controls (0), 60 and 120 mg/kg dose groups, respectively. The only monochlorobenzene dosed group found to be significantly different from controls in survival rates was the 30 mg/kg male group ($p=0.031$). Histological findings of neoplasms will be discussed in Section 7.3.5. Carcinogenicity. No statistically significant increased or decreased incidences in site-specific tumors or non-neoplastic pathology were found in either the male or female mice (NTP, 1983b).

The rat study revealed no monochlorobenzene-related clinical signs of toxicity during the 104-week study period (exposure duration 103 weeks).

The only differences noted in body weights during this study were increased body weights in the monochlorobenzene-treated females during the second study year. The only significant differences in survival rates were observed in the male 120 mg/kg dose group which had significantly reduced survival rates (p=0.014 as compared with vehicle control). The survival rates during this study were 68% (34/50), 78% (39/50), 64% (32/50) and 52% (26/50) in male rats and 74% (37/50), 58% (29/50), 60% (30/50) and 62% (31/50) in female rats for the untreated controls, vehicle controls (0), 60 and 120 mg/kg dose groups, respectively. Histological findings of neoplasms will be discussed in Section 7.3.5. Carcinogenicity. Histological evaluation of liver tissue provided equivocal evidence for mild monochlorobenzene-induced hepatocellular necrosis. The control rat livers were observed to have more basophilic cytoplasmic changes than the monochlorobenzene-treated rats (NTP, 1983b).

7.3.4. **Mutagenicity.** Studies of the mutagenicity of monochlorobenzene have yielded mixed results, with the greater proportion of the studies being negative (Table 7-5).

7.3.5. **Carcinogenicity.** The only study available on the assay of monochlorobenzene for carcinogenic potential is one conducted by the National Toxicology Program (NTP), 1983. This study was carried out with F344/N rats and with $B6C3F_1$ mice. In both the rat and mouse tests the compound was prepared in corn oil and administered by gavage, on a 5 day/week schedule, for 103 weeks to groups of 50 male and 50 female animals at each dose. The doses selected in the rat study were 60 and 120 mg/kg/day for both male and female animals while in the case of mice the females received 60 or 120 mg/kg/day but the males received 30 or 60 mg/kg/day. The test compound was 99% pure.

TABLE 7-5

Mutagenicity Testing of Monochlorobenzene

Test System	Metabolic Activation	Concentration	Result	Reference
Asperigillus nidulans	-	200 μg/mℓ	negative	Prasad, 1970
Salmonella strains TA1535, TA1537, TA1538, TA92, TA98, TA100	±	0.1-0.5 μℓ/plate	negative	Simmon et al., 1979
Salmonella typhimurium strains	±	100 μg/plate	negative	Merck, 1978
Salmonella typhimurium strains	±	150-3000 μg/plate	negative	DuPont, 1977
Saccharomyces cerevisiae	±	0.05-6%	positive	Simmon et al., 1977
Saccharomyces cerevisiae	±	0.01-5 μℓ/plate	negative	Monsanto, 1976
Mouse lymphoma L5178Y (forward mutation of TK)	- +	0.001-0.1 μℓ/mℓ 0.0001-0.01 μℓ/mℓ	negative	Monsanto, 1976
DNA repair:				
Escherichia coli ($polA^+/polA^-$)	-	10-20 μℓ/plate	negative	Simmon et al., 1979
Bacillus subtilis (rec^-/rec^+)	-	10-20 μℓ/plate	negative	Simmon et al., 1979
Streptomyces antibioticus		NR	positive	Keskinova, 1968

NR = Not reported

7.3.5.1. RAT STUDY -- In the case of the F344/N rats, dose selection was made as a result of observations in the 13 week subchronic study. In the 13-week study doses of 60, 125, 250, 500 and 750 mg/kg for each day of dosing were used. There was essentially 100% survival among both sexes up to and including the 250 mg/kg groups; at 500 mg/kg/day the mortality was 30-40% and at the top dose, 750 mg/kg, the mortality was 80-90%. Among male rats the body weight gain was depressed by 12% relative to the controls for 250 mg/kg or higher, and 12% or greater among females at the 500 and 750 mg/kg groups. Histopathology was carried out on all the major organs for rats in the 500 and 750 mg/kg groups, on kidneys, bone marrow and liver for animals in the 250 mg/kg groups and only on liver and kidney in the 125 mg/kg groups. Hepatic necrosis was seen in 2/10 males and 1/10 females at 250 mg/kg and occurred with greater frequency in the higher dose groups. Other toxic manifestations including nephrotoxicity, lymphoid and myeloid depletion of spleen, bone marrow and thymus, and abnormal porphyrin metabolism occurred at the 500 mg/kg and higher doses. These findings on survival, weight decrement and pathology formed the basis for selection of the 60 and 120 mg/kg dosages for the 2-year study in the rats.

In the 2-year study survival of males at the 120 mg/kg groups was significantly reduced when compared with vehicle but not with untreated controls. It is not clear whether the accidental deaths were censored from this evaluation. There were four such accidental deaths among the high dose males, two at the low dose and one in a vehicle control male. Among females there were seven accidental vehicle control deaths, four at the low dose and two at the high dose.

The histopathology review in the 2-year study resulted in conflicting interpretation by different pathologists with respect to hepatocellular necrosis, hepatocellular basophilic cytoplasmic changes and granulomatous inflammation. The findings of the 2 different reviewers are given, as they appear in the NTP report, in Table 7-6. It is not clear whether these differing interpretations of non-neoplastic lesions have any bearing on the single set of results reported for neoplastic nodules and carcinomas (Table 7-7). In males no carcinomas were observed in the treated groups, but there was a statistically significant increase in neoplastic nodules in the high dose group and a marginally significant dose-response trend. Neither neoplastic nodules nor hepatocellular carcinoma were increased in female rats.

In this study interstitial cell tumors of the testis showed a significant positive trend and the incidence in the high dose group was significantly different from the vehicle control in the life-table test. These statistics are, however, without biological significance since the untreated controls had incidence of 100%. The vehicle control had 93.7% incidence and the low dose 97.7% while the high dose had 100%.

Both pituitary tumors (adenomas in female rats and combined adenomas and carcinomas in male rats) and endometrial stromal polyps of the uterus showed significant negative trends.

In the F344/N rats, therefore, the significant increase in neoplastic nodules in the liver of male animals at the 120 mg/kg/day dose group provides some evidence for tumorigenicity of monochlorobenzene.

7.5.3.2. **MOUSE STUDY** -- The choice of dose for the chronic study in mice was based on the results of a 13 week subchronic test. In the 250 mg/kg group of the 13-week study 4/9 males died (time of death was 1 week for one animal and 10 weeks for the other three animals), and there was a 20% weight decrement compared to controls, showing clear evidence of toxicity. In the next lower test group (125 mg/kg), no males died and the

TABLE 7-6

Nonneoplastic Lesions in F344 Rats Given Chlorobenzene by Gavage for 2 Years*

	Males				Females			
	UC	VC	Low Dose	High Dose	UC	VC	Low Dose	High Dose
Number of livers examined	50	50	49	49	49	50	50	50
First Reading								
Hepatocellular necrosis	2	1	4	5	0	0	1	7
Cytoplasmic basophilia change	25	27	6	3	38	27	18	10
Inflammation	9	9	3	0	23	21	11	11
Second Reading								
Hepatocellular necrosis	3	2	5	1	1	1	2	1
Cytoplasmic basophilia change	28	40	12	12	43	34	26	18

*Source: NTP draft, 1983b

UC = Untreated controls; VC = vehicle controls

TABLE 7-7

Statistical Comparisons of Liver Tumors in Male Rats Treated with Chlorobenzene and Vehicle Controls*

	Untreated Control	Vehicle Control	60 mg/kg	120 mg/kg
Neoplastic nodule				
Overall	4/50(8%)	2/50(4%)	4/49(8%)	8/49(16%)
Adjusted	10.4%	4.5%	12.5%	29.3%
Terminal	2/34(6%)	0.39(0%)	4/32(13%)	7/26(27%)
Life Table		P=0.005	P=0.255	P=0.010
Incidental Tumor Test		P=0.011	P=0.290	P=0.021
Cochran-Armitage Trend, Fisher Exact Tests		P=0.027	P=0.329	P=0.043
Carcinoma				
Overall	0.50(0%)	2/50(4%)	0/49(0%)	0/49(0%)
Adjusted	0.0%	5.1%	0.0%	0.0%
Terminal	0/34(0%)	2/39(5%)	0/32(0%)	0/26(0%)
Life Table		P=0.139N	P=0.283N	P=0.331N
Incidental Tumor Test		P=0.139N	P=0.283N	P=0.331N
Cochran-Armitage Trend, Fisher Exact Tests		P=0.098N	P=0.253N	P=0.253N
Neoplastic Nodule or Carcinoma				
Overall	4/50(8%)	4/50(8%)	4/49(8%)	8/49(16%)
Adjusted	10.4%	9.4%	12.5%	29.3%
Terminal	2/34(6%)	2/39(5%)	4/32(13%)	7/26(27%)
Life Table		P=0.033	P=0.532	P=0.048
Incidental Tumor Test		P=0.054	P=0.570	P=0.083
Cochran-Armitage Trend, Fisher Exact Tests		P=0.121	P=0.631	P=0.168

*Source: NTP draft, 1983b

average weight was within 6% of the controls, The histopathology review of the 125 mg/kg group showed one male mouse with hepatic necrosis. There was also one male with hepatic necrosis in the 60 mg/kg/day group. On the basis of these data one can conclude that doses up to 120 mg/kg probably could have been tolerated in the chronic study of male mice, whereas only 60 and 30 mg/kg were actually used. However, the NTP draft document (dated Feb. 28, 1983) stated that "doses of 30 and 60 mg/kg were selected for male mice because of a perceived greater susceptibility of this sex to the toxic effects of chlorobenzene".

The survival and body weight data in males during the chronic study also suggest that larger doses could have been tolerated. Body weights in both dose groups and survival in the high dose group were comparable to controls. Although survival was reported to be significantly reduced in the low dose group (30 mg/kg), two animals that died had foreign material in the lungs, suggesting that gavage errors rather than toxicity was responsible for the reduced survival in that group. These two animals were included as deaths from natural causes.

After histopathological analysis the NTP found that both tumor incidence and non-neoplastic pathology were comparable to controls at all sites in both male and female treated groups. The test in mice therefore provided no evidence of carcinogenicity at doses as high as 60 mg/kg. Note, however that the F344/N rats did not develop neoplastic nodules until the dose was as high as 120 mg/kg.

In summary, the evidence for the carcinogenicity of monochlorobenzene from the NTP study on F344/N rats and $B6C3F_1$ mice consists of the finding of a significant increase in neoplastic nodules in the liver in male rats that received 120 mg/kg, for 5 days/week for 2 years. If the IARC criteria for classifying carcinogens were used, this evidence would be characterized as limited to inadequate in animals. Since there is no human evidence relating to carcinogenicity, the overall IARC classification is category 3, and no conclusions can be made concerning the carcinogenicity of monochlorobenzene in humans.

7.3.6. Reproductive and Teratogenic Toxicity. Monsanto Company (1978) reported effects on the gonads of dogs exposed to monochlorobenzene vapor at 0, 0.76, 1.47 and 2.0 mg/ℓ for 6 hours/day, 5 days/week for a total of 62 exposures. Two of four male dogs in the high dose group developed bilateral atrophy of epithelial tissue in the seminiferous tubules. These effects are consistent with an earlier Monsanto (1967a) study where four male and four female dogs were orally given monochlorobenzene at 0.025, 0.050 and 0.250 mg/kg/day doses for 13 weeks. Three of the four male dogs in the high dose group had decreased spermatogenesis and this group also had tubular atrophy and epithelial degeneration.

Rats exposed to monochlorobenzene vapor at 0, 0.76, 1.47 and 2.0 mg/ℓ for 6 hours/day, 5 days/week for a total of 62 exposures showed less definite gonadal responses (Monsanto, 1978). The 2.0 mg/ℓ exposed female rats exhibited significantly higher gonad-to-body-weight ratio when compared to control females.

No studies regarding the teratogenicity of monochlorobenzenes were available for review.

7.4. INTERACTIONS

Monochlorobenzene produces a variety of alterations in enzyme function and would, therefore, be expected to influence the metabolism and toxicity of a variety of compounds. Shelton and Weber (1981) investigated the hepatotoxicity of a mixture of CCl_4 and monochlorobenzene (1:38 molar ratio) to male CF-1 mice. The mixture was given intraperitoneally in corn oil (0.01 mℓ/g bw). For the mixture of CCl_4 and monochlorobenzene, the plasma analine aminotransferase dose-response curve did not deviate from that predicted on the basis of dose addition.

7.5. SUMMARY

Acute exposure to monochlorobenzene by inhalation causes sensory irritation of the respiratory system after a few minutes; exposure for several minutes to several hours causes narcosis and central nevous system depression, which can result in death. It is also toxic by the oral or parenteral routes. Systemic effects of acute toxic doses include kidney damage. Subchronic inhalation exposure at 1.0 mg/m³ (continously for 60 days) causes neurotoxic effects in rats, an increase in blood cholinesterase and abnormal chronaxia of the muscles. Repeated exposure of rats to monochlorobenzene at 250 ppm (1157 mg/m³) causes slight changes in the liver, kidneys and adrenal cortex. Repeated oral dosing of rats or dogs (100-200 mg/kg/day) causes some toxic manifestation in the liver and kidneys.

Gavage administration of monochlorobenzene to mice and rats 5 times/week for 13 weeks resulted in increased mortality in the higher dose groups ($\geq$250 mg/kg), urinary porphyria and dose-dependent injury to the liver, kidney, bone marrow, spleen and thymus. A set of similar studies were conducted in mice and rats for 2 years and resulted in some increased mortality in the male monochlorobenzene exposed groups when compared with controls. Only equivocal evidence for mild monochlorobenzene-induced hepatocellular necrosis was found in rats.

Although one study in Streptomyces found monochlorobenzene to induce reversion to vitamin B_1 prototrophy and one study in Saccharomyces cerevisiae showed increased mitotic crossing over (indication of DNA damage), several other studies with bacterial, fungal and mammalian tissue culture systems were negative. The carcinogenic activity of monochlorobenzene was tested in the NTP bioassay program in two rodent species at doses of 60 and 120 mg/kg bw/day in male and female rats and female mice, and at 30 and 60 mg/kg bw/day in male mice. Carcinogenicity was not demonstrated for monochlorobenzene by this study.

Repeated exposures to monochlorobenzene at 2.0 mg/ℓ (vapors) or 0.250 mg/kg/day (oral) were found to cause atrophy of the epithelial tissue of the seminiferous tubules and decreased spermatogenesis in male dogs and rats and increased gonad weight/body weight ratios in female rats.

8. DICHLOROBENZENES

The 1983 annual production of dichlorobenzenes in the United States is estimated to be between 46.7 and 50.2 million kilograms (U.S. EPA, 1983). These materials are used primarily as fumigants, insecticides, solvents, dye carriers and space deodorants (Hawley, 1977). Measurable levels of dichlorobenzenes have been reported in ambient urban and rural air and in samples of indoor air, in ground, surface and wastewater and in runoff from hazardous waste sites (see Section 4.3.). Residues have been found in fish and other aquatic organisms and in samples of human fat, blood, breath and urine (see Section 4.3.3.). Human exposure is most likely through the inhalation of air and ingestion of contaminated food and drinking water.

8.1. PHARMACOKINETICS

8.1.1. Absorption. The dichlorobenzenes have low water solubility and high lipid solubility and therefore are likely to diffuse through most biological membranes, including the surfaces of the lungs and gastrointestinal tract and the skin. The absorption of dichlorobenzenes by humans is indicated by poisonings that have resulted from exposures by inhalation or ingestion. Quantitative studies of the absorption of dichlorobenzenes in humans and animals are lacking. The available data indicate that absorption does occur fairly rapidly through the lungs and gastrointestinal tract. Skin absorption has not been tested adequately.

Twenty-three cases of poisoning by dichlorobenzenes have been reported in the available literature and provide evidence of human absorption (Downing, 1939; Perrin, 1941; Petit and Champeix, 1948; Sumers et al., 1952; Weller and Crellin, 1953; Cotter, 1953; Hallowell, 1959; Frank and Cohen, 1961; Gadrat et al., 1962; Nalbandian and Pearce, 1965; Girard et al., 1969; Campbell and Davidson, 1970; Ware and West, 1977; Harden and Baetjer, 1978).

Of these cases, 5 involved 1,2-dichlorobenzene as the principal or significant source of exposure and 11 involved 1,4-dichlorobenzene. Inhalation was the primary route of exposure for 17 of the cases and 3 involved ingestion. Three of the cases also mentioned previous dermal exposures that may have contributed to the reported intoxication.

Hawkins et al. (1980) exposed ten female CFY rats to a nominal air concentration of 1000 ppm of 1,4-dichloro-[^{14}C]benzene, 3 hours/day for up to 10 days. In parallel experiments, groups of 20 female CFY rats were given daily oral or subcutaneous doses of 1,4-dichloro-[^{14}C]benzene dissolved in sunflower oil. Twenty-four hour tissue concentrations of ^{14}C were similar for each treatment route, with the highest concentrations occurring in the fat, kidneys, liver and lungs. 1,4-Dichlorobenzene appears to be well absorbed through both the lungs and gastrointestinal tract; however, no quantitative measures of absorption were attempted.

Kimura et al. (1979) administered 200 or 800 mg/kg of 1,4-dichlorobenzene in corn oil orally to male Wistar rats and monitored the appearance of the chemical in blood, adipose, kidney, liver, lung, heart and brain tissue. At the first time point, 30 minutes after dosing, all these tissues contained measurable amounts of dichlorobenzene, with liver and adipose tissue having 2 and 10 times the concentrations seen in the blood, respectively.

Three other studies have suggested that dichlorobenzenes can be almost completely absorbed from the gastrointestinal tract, even when present in low doses. Azouz et al. (1955) dosed chinchilla rabbits intragastrically with 1.5 g 1,4-dichlorobenzene/rabbit in olive oil and did not detect any of the compound in the feces, implying that under the conditions of this study, a total absorption had occurred. Hawkins et al. (1980) administered a single dose of labeled 1,4-dichlorobenzene (250 mg/kg) to rats with cannulated bile ducts, which prevented fecal excretion of the metabolized compound. During the following 24 hours, 9% of the label was present in the feces, representing the unabsorbed portion of the dose. 1,2-Dichlorobenzene and other organic contaminants of water were administered to rats in the diet at levels of 0.4-2 mg/kg/day (Jacobs et al., 1974a,b). The accumulation of the compound in several tissues indicated that absorption occurs after the ingestion of low levels of 1,2-dichlorobenzene.

Riedel (1941) has investigated the dermal absorption of 1,2-dichlorobenzene in rats. No quantitative measurements were made; however, five applications were lethal when the compound was applied directly to a 10 cm^2 area of abdominal skin.

Absorption of dichlorobenzenes can occur through the lungs, skin and gastrointestinal tract. Quantitative studies of absorption through the lungs and gastrointestinal tract are lacking, as well as studies on dermal absorption.

8.1.2. Distribution. The low water and high lipid solubility of the dichlorobenzenes enable their diffusion through membranes and therefore enhance their tissue distribution. Several studies in animals have quantified the degree and time course of the distribution of dichlorobenzenes after inhalation and ingestion and indicate rapid distribution to blood, adipose, kidney, liver, lung, heart, brain and muscle tissue.

Hawkins et al. (1980) investigated the distribution and excretion of 1,4-dichlorobenzene in adult female CFY rats (derived from Sprague-Dawley rats) after repeated inhalation, oral and subcutaneous doses. Radioactively labeled 1,4-dichlorobenzene was administered by exposing groups of 10 rats to the compound at an atmosphere of 1000 ppm for 3 hours/day for 10 consecutive days or by oral or subcutaneous doses of 250 mg/kg/day for 10 days.

The investigators reported tissue concentrations after 2, 4, 6, 8 and 10 doses. Radiolabel was widely distributed following each route of administration, with the highest concentrations occurring in fat, kidney, liver and lungs (Table 8-1).

Kimura et al. (1979) also provided information on the distribution of 1,4-dichlorobenzene. Tissue levels of the compound were monitored at intervals from 30 minutes to 120 hours in male Wistar rats given a 200 mg/kg oral dose in corn o'l after a 16-hour fast. At the first interval, fat and liver levels were 10 and 2 times the blood levels (~9 μg/mℓ), respectively, with lower concentrations of dichlorobenzene appearing in all of the other tissues examined (kidney, lung, heart and brain). Levels in fat, kidney and liver tissue peaked between 6 and 12 hours (at ~50, 2 and 0.5 times blood levels, respectively) and thereafter, decreased along with the levels in the other tissues. After 48 hours, concentrations of 1,4-dichlorobenzene were below the detection limit in all tissues examined, except for fat tissue, which had detectable levels equal to approximately one-fiftieth of peak concentrations at 120 hours post-administration.

Tissue distribution after subchronic feeding of 1,2-dichlorobenzene was investigated by Jacobs et al. (1974a,b), who administered the compound in a mixture of other organic chemicals at doses of 0.4, 0.8 and 2 mg/kg/day for 4-12 weeks to rats. A dose-related accumulation of 1,2-dichlorobenzene was reported in abdominal and renal adipose tissue to an extent greater than that seen in liver, heart and blood tissues.

Studies of tissue distribution of dichlorobenzenes after repeated inhalation exposure and single and subchronic oral exposure indicate that the chemicals appear in all of the major tissues soon after dosing with the

TABLE 8-1

Tissue Concentrations of 1,4-Dichlorobenzene in Adult Female CFY Rats[a,b,c] (ppm)

Number of Doses	Liver			Kidneys			Lungs			Fat		
	Inhalation[c]	Oral	Subcutaneous	Inhalation	Oral	Subcutaneous	Inhalation	Oral	Subcutaneous	Inhalation	Oral	Subcutaneous
2	14	11	21	24	27	30	9	7	18	418	218	372
4	22	18	22	40	29	32	12	13	12	579	369	302
6	28	14	24	43	23	47	11	10	14	597	170	269
8	16	15	21	28	18	41	10	11	21	433	131	554
10	18	9	20	27	16	32	10	9	17	337	257	383

[a] Source: Adapted from Hawkins et al., 1980

[b] Female rats were exposed daily to 1,4-dichlorobenzene via: inhalation, 1000 ppm for 3 hours/day; oral, 250 mg/kg in sunflower oil; subcutaneously, 250 mg/kg in sunflower oil and killed 24 hours after last dosing.

[c] Values represent the average from two animals

highest levels, in descending order, in adipose, kidney, liver and lung tissue. Peak concentrations are reached in all tissues within 4-12 hours, followed by almost total elimination (Section 8.1.4.). The pattern of distribution after inhalation, subcutaneous and oral administration is similar.

8.1.3. **Metabolism.** The metabolism of the dichlorobenzenes has been investigated primarily in rabbits and rats; few data were available on metabolism in humans. Several studies have shown the primary metabolites to be dichlorophenols that are conjugated with glucuronic and sulfuric acids and excreted. Formation of the dichlorophenols from 1,2- and 1,3-dichlorobenzene appears to involve epoxidase and arene oxide intermediates.

Azouz et al. (1955) studied the metabolism of 1,2- and 1,4-dichlorobenzene in rabbits given oral doses of 500 mg/kg. The compounds were metabolized primarily through oxidation to 3,4-dichlorophenol (from 1,2-dichlorobenzene) and 2,5-dichlorophenol (from 1,4-dichlorobenzene) and excreted in the urine in the form of glucuronic and sulfuric acid conjugates. Minor metabolites of 1,2-dichlorobenzene included the 4,5- and 3,4-dichlorocatechols and 3,4-dichlorophenylmercapturic acid; a minor metabolite of 1,4-dichlorobenzene is 2,5-dichloroquinol. Metabolism and complete elimination required 5-6 days for 1,2-dichlorobenzene and >6 days for 1,4-dichlorobenzene. Kimura et al. (1979) found similar results in male Wistar rats; oral administration of 200 or 800 mg/kg of 1,4-dichlorobenzene resulted in the formation of one major metabolite, 2,5-dichlorophenol, and two minor sulfur-containing metabolites (<0.03% of the total dose). These two compounds, identified as 2,5-dichlorophenol methoxy sulfoxide and 2,5-dichlorophenol methyl sulfone, were excreted over a 5-day period and were detected in blood, fat, kidney and liver tissues.

Hawkins et al. (1980) investigated the metabolism of radiolabeled 1,4-dichlorobenzene in female CFY rats after repeated inhalation (1000 ppm for 3 hours/day), oral or subcutaneous (250 mg/kg/day) exposures. After dosing for 10 consecutive days, all of the label was metabolized and eliminated within 192 hours (8 days). Both routes of exposure resulted in similar urinary and biliary metabolites, primarily 2,5-dichlorophenol sulfate (46-54% of the total excreted) and 2,5-dichlorophenol glucuronide in the urine (31-34%) and bile (30-42%). Two minor metabolites were identified as dihydroxydichlorobenzene and a mercapturic acid of 1,4-dichlorobenzene.

Parke and Williams (1955) studied the metabolism of 1,3-dichlorobenzene in rabbits and found dichlorophenol to be the major metabolite, accounting for 40% of the total amount of excreted metabolites. 2,4-Dichlorophenylmercapturic acid and 3,5-dichlorocatechol were also detected. No analogous studies have been conducted in humans although Pagnotto and Walkley (1966) reported that 2,5-dichlorophenol was present in the urine of men occupationally exposed to 1,4-dichlorobenzene by inhalation. Several studies have indicated that the dichlorobenzenes are also capable of inducing hepatic microsomal enzymes and enhancing the synthesis of porphyrins. Rimington and Ziegler (1963), Poland et al. (1971) and Ariyoshi et al. (1981) have reported the induction of δ-aminolevulinic acid synthetase in rats by daily doses of 250-1000 mg/kg of dichlorobenzenes. This enzyme is involved in the rate-limiting step of the synthesis of porphyrins and its induction is necessary for an increase in the activity of cytochrome P-450 and other xenobiotic metabolizing enzymes.

8.1.3.1. **COVALENT BINDING** -- Metabolism of dichlorobenzenes results in the formation of reactive species, which may bind covalently to cellular macromolecules. This binding may lead to some toxic effects of the dichlorobenzenes. Reid and Krishna (1973) studied the relationship between the binding of metabolites of halogenated aromatic hydrocarbons and the induction of hepatic necrosis. Labeled bromobenzene, 1,2- and 1,4-dichlorobenzene, as well as other aromatic compounds, were injected intraperitoneally into Sprague-Dawley rats in 0.5 mmol/kg doses. A correlation between covalent binding of bromobenzene to protein and the time course and degree of hepatic centrolobular necrosis was established. 1,2-Dichlorobenzene also was found to bind to liver protein and the binding was enhanced by pretreatment with phenobarbital. 1,4-Dichlorobenzene showed little binding. The authors interpreted these results to mean that the hepatic injury induced by 1,2-dichlorobenzene was a result of the binding to protein of reactive intermediates whose synthesis was increased by the induction of hepatic xenobiotic-metabolizing enzymes. 1,4-Dichlorobenzene is less hepatotoxic than 1,2-dichlorobenzene and does not bind to the same degree. Similar results were obtained for the bronchiolar necrosis occurring in lung tissue (Reid et al., 1973).

8.1.4. Excretion. Hawkins et al. (1980) measured the excretion of ^{14}C in female CFY rats following whole body exposure by inhalation (1000 ppm, 3 hours/day, 2-10 days), by oral (250 mg/kg/day) or subcutaneous (250 mg/kg/day) routes of 1,4-dichloro[^{14}C]benzene. Excretion occurred primarily via the urine (91-97% of the total excreted) over a 5-day period after repeated dosings had stopped, with only minor amounts occurring in the feces and expired air. Following a single oral dose to bile-duct-cannulated rats, 46-63% of the ^{14}C excreted during the first 24 hours was found in the bile. This implies that enterohepatic recirculation occurs to a major extent with this compound. Excretion seemed to involve a rapid initial phase followed by a slower extended excretion phase.

Kimura et al. (1979) observed similar excretion patterns in male Wistar rats. They suggested that the prolonged excretion of 1,4-dichlorobenzene resulted from the release of unmetabolized material from fatty depots and the slow excretion rates for 2,5-dichlorophenyl methyl sulfone and 2,5-dichlorophenyl methyl sulfoxide, two metabolites of 1,4-dichlorobenzene.

Azouz et al. (1955) compared the excretion of 1,2-dichlorobenzene and 1,4-dichlorobenzene given to chinchilla rabbits by stomach tube in an olive oil solution. Excretion rates were not determined; however, the excretion of the ortho isomer appeared to be complete within 5-6 days after dosing. With the para isomer. appreciable excretion of metabolites still occurred after 6 days. Excretion of the meta isomer in chinchilla rabbits was found to be virtually complete within 5 days after dosing by stomach tube using an olive oil solution (Parke and Williams, 1955). One study has suggested that similar metabolic products occur in humans. Pagnotto and Walkley (1966) reported that the appearance of dichlorophenol in the urine of workers that were exposed to 1,4-dichlorobenzene began soon after exposure, peaked at the end of the shift and continued for several days.

8.1.5. **Summary.** The available data for rats, rabbits and humans indicate that the dichlorobenzenes are absorbed through the lungs, gastrointestinal tract and intact skin, though actual determinations of absorption rates were not located. Once absorbed through either inhalation or ingestion, the dichlorobenzenes are rapidly distributed to many tissues, including blood, adipose, kidney, liver, lung, heart, brain and muscle tissues. Distribution is primarily to adipose tissue, which has initial levels 10-32 times the blood concentrations and to lung and kidney tissues to a greater extent than liver, muscle and plasma. Single-dose and repeated exposures by both inhalation and ingestion show similar patterns of distribution. Elimination of the dichlorobenzenes and their metabolites occurs within 5-6 days after exposure, although elimination from adipose tissue is slowest and 1,2-dichlorobenzene and metabolites are eliminated slightly more rapidly than 1,4-dichlorobenzene. The dichlorobenzenes are primarily metabolized by hydroxylation to their respective dichlorophenols, which are excreted in the urine in the form of glucuronic and sulfuric acid conjugates. Some metabolites are excreted in the bile, although the majority are then reabsorbed by the enterohepatic pathway and reexcreted in the urine. Intermediates of the metabolism of 1,4-dichlorobenzene, possibly arene oxides, bind to liver protein and may be involved in the induction of hepatotoxicity.

8.2. EFFECTS ON HUMANS

8.2.1. Occupational Studies. One occupational study was available for review. Zapata-Gayon et al. (1982) performed chromosomal studies on 8 males and 18 females who were accidentally exposed to vapors of 1,2-dichlorobenzene for four 8-hour workdays. Karyotypes of cells from samples of peripheral blood from the exposed subjects were compared with those obtained from 16 controls (8 male, 8 female). Exposed subjects and controls had similar occupational histories: all worked in a biological laboratory performing electron microscope and tissue culture work. Recent history of prolonged X-ray exposure, infection or exposure to other toxic chemicals was not found among the subjects. The exposure to 1,2-dichlorobenzene, which resulted from its use as a pest control in the basement of a one-story building, caused dizziness, headache, fatigue, nausea and eye and nose irritation in all but four of the subjects. Karyotype analysis, performed independently by two cytogeneticists, found that the total number of altered cells, identified as having clastogenic chromosomal alterations, was greater in the exposed versus control groups (8.9 vs. 2.0%, $p<0.001$, multiple chi-square tests). In addition, the total number of single chromosomal breaks (6.2 vs. 0.9%, $p<0.001$) and double breaks (6.4 vs. 1.6%, $p<0.001$) were different. A follow-up study was conducted on 15 of the original exposed cases 6 months after the initial exposure. The investigators reported that the number of altered cells and single breaks was not significantly different ($p<0.05$) from the original control frequencies, but that the number of double breaks was increased (3.7 vs. 1.6%, $p<0.01$). No analysis of the number of altered cells/person was performed, although these data, reported in the form of a histogram, showed distinct differences (Table 8-2). The investigators also noted the presence of other aberrations (polyploidy and ring formation) that were not statistically significant.

8.2.2. Case Studies. Numerous case studies have been reported in the literature involving both long-term occupational exposure and accidental or deliberate acute exposure. Of these cases (a total of 23), 17 have involved exposure primarily through inhalation, 3 through ingestion and 3 most likely through dermal absorption. The principal agent in 16 of these exposures was 1,4-dichlorobenzene; the remainder involved 1,2-dichlorobenzene or mixtures of all three dichlorobenzene isomers. In all of these cases, toxic effects have been reported in one or more of the following: liver; blood, including reticuloendothelial system; central nervous system; and respiratory tract. A summary of these reports, which were compiled in U.S. EPA (1980c) with the exception of Hardin and Baetjer (1978), is given in Table 8-3.

Two surveys of the health of workers occupationally exposed to 1,4-dichlorobenzene during its manufacture have been reported. Hollingsworth et al. (1956) reported that periodic medical examinations showed no evidence of injury or adverse changes in hematology or eye lenses in workers

TABLE 8-2

Chromosomal Alterations in Persons Accidentally Exposed to 1,2-Dichlorobenzene*

Number of Altered Cells per Person	Percentage Control (n=16)	 Exposed (n=22)
0-1	83	5
2-3	19	35
4-5	0	29
>6	0	31

*Source: Adapted from Zapata-Gayon et al., 1982

TABLE 8-3

Case Reports Involving Dichlorobenzenes (DCB)*

Chemical/Mixture	Subject and Exposure	Effects	Reference
1,2-DCB (vapor)	Sewage workers; occupational; inhalation; effluent from dry cleaning establishment	Eye and upper respiratory tract irritation; vomiting	Dupont, 1938
1,2-DCB solvent mixtures: 80% 1,2-DCB; 15% 1,4-DCB; 2% 1,3-DCB	Male, 40 years; occupational; use of solvent to clean equipment; chronic daily exposure probably via inhalation of vapors, and dermal absorption from clothing	Weakness, fatigue; peripheral lymphadenopathy; chronic lymphoid leukemia	Girard et al., 1969
1,2-DCB solvent mixure: 95% 1,2-DCB; 5% 1,4-DCB	Female, 18 years; occupational; chronic daily inhalation exposure to vapors as pressing-ironing worker	Severe acute hemolytic anemia; leukocytosis, polynucleosis; fatigue, nausea, headache; bone marrow hyperplasia; possible inherent predisposing factor	Gadrat et al., 1962
1,2-DCB and other chlorobenzenes	Male, 60 years; occupational; filling barrels with 1,2-DCB and other chlorobenzenes; chronic inhalation of vapors (last 3 years); perhaps also skin contact	Anemia	Girard et al., 1969
1,2-DCB in a mixture	Male, 47 years; occupational; handling window sashes dipped in mixture; chronic skin contact (also inhalation)	Contact eczematoid dermatitis on hands, arms, face, erythema, edema; bullae in response to skin test	Downing, 1939
1,2-DCB (37% in solution)	Female, 15 years; nonoccupational; chronic repeated dermal contact from compulsive use of cleaning solution on clothing	Acute myeloblastic leukemia progressing to 100% leukoblastosis, hemorrhage and death	Girard et al., 1969
1,2-DCB solvent mixture: 80% 1,2-DCB; 15% 1,4-DCB; 2% 1,3-DCB	Female, 55 years; nonoccupational; chronic repeated inhalation of vapors from use of solution to clean clothes; 1-2 ℓ/year	Acute myeloblastic leukemia	Girard et al., 1969
1,4-DCB primarily	Female, 30 years; occupational; chronic inhalation and dermal contact from 2 years of selling mothballs and insecticide products containing 1,4-DCB	Weakness, nausea, splenomegaly, "severe hepatocellular derangement, and ensuing portal hypertension" with esophageal varices	Sumers et al., 1952

TABLE 8-3 (cont.)

Chemical/Mixture	Subject and Exposure	Effects	Reference
1,4-DCB primarily	Female, 34 years; occupational; chronic inhalation from demonstrating 1,4-DCB products in booth in department store	Malaise, then acute nausea, vomiting, headache, jaundice, hepatomegaly, splenomegaly, esophageal varices, and hemorrhoids; subacute yellow atrophy and cirrhosis of liver	Cotter, 1953
1,4-DCB	Male, 52 years; occupational; chronic inhalation of high vapor levels in a fur warehouse	Weakness, nausea, hematemesis, jaundice, emaciation, petechia, hemorrhages; hepatomegaly, splenomegaly, hemorrhoids; proteinuria, bilirubinuria; hematuria; anemia, leukopenia; subacute yellow atrophy of liver	Cotter, 1953
1,4-DCB primarily	Female, 19 years; occupational; crushing, pouring, sieving, filling containers; poor ventilation; chronic inhalation of vapors	Marked asthenia, dizziness, weight loss; anemia and reactional leukocytosis	Petit and Champeix, 1948
1,4-DCB	Female, occupational; casting 1,4-DCB in molds; chronic inhalation	Severe anemia	Perrin, 1941
1,4-DCB	Male, 20 years and workmates; occupational; 1,4-DCB manufacturing activities, 1-7 months of exposure; inhalation	Weight loss, exhaustion, and decreased appetite; methemoglobinemia and other blood pathologies	Ware and West, 1977
1,4-DCB	Male, 62 years; nonoccupational; used "moth killer" product in bathroom at home, chronic inhalation of vapors, and wearing of impregnated clothing (possible skin exposure)	Asthenia, dizziness; anemia, hypogranulocytosis (similar to cases of intoxication by benzene)	Perrin, 1941
1,4-DCB	Female, 36 years; nonoccupational; use of commercial moth killer in home (presumably inhalation of vapors)	Acute illness with intense headache, profuse rhinitis, periorbital swelling	Cotter, 1953
1,4-DCB	Male, 60 years; nonoccupational; 3-4 months exposure to "moth gas vapor" in home	Headache; weight loss; diarrhea; numbness; clumsiness; jaundice; enlarged liver; anemia; neutropenia; ascites; death; acute yellow atrophy of liver noted at autopsy	Cotter, 1953

TABLE 8-3 (cont.)

Chemical/Mixture	Subject and Exposure	Effects	Reference
1,4-DCB	Female, wife of above, nonoccupational; prolonged severe exposure to "moth gas vapor"	Gradual loss of strength and weight, then abdominal swelling and jaundice before acute illness; elevated temperature and pulse, dilated vessels, swollen liver, toxic granulocytosis; died 1 year later; acute yellow atrophy of liver, Laennec's cirrhosis and splenomegaly noted at autopsy	Cotter, 1953
1,4-DCB	Female, 53 years; nonoccupational; used moth eradicator product heavily in home for 12-15 years, odor always apparent; chronic inhalation of vapor	Chronic progressive cough and dyspnea with mucoid sputum, wheezing, fatigue, diminished breath sounds and rales; abnormal lung field on X-ray; fibrotic, rubbery lung with histologic changes; diagnosis: pulmonary granulomatosis	Weller and Crellin, 1953
1,4-DCB	Male, 3 years; nonoccupational; played with canister of demothing crystals, spreading on floor, handling; ingestion, likely acute	Listlessness, jaundice, oliguria, methemoglobinuria and other urine abnormalities, anemia, hypothermia; diagnosis: acute hemolytic anemia	Hallowell, 1959
1,4-DCB	Female, 21 years; nonoccupational; ingestion during pregnancy (pica) of toilet air freshener blocks at rate of 1-2/week	Fatigue, anorexia, dizziness, edema of ankles; hypochromic microcytic anemia; bone marrow normoblastic hyperplasia; diagnosis: toxic hemolytic anemia; complete recovery	Campbell and Davidson, 1970
1,4-DCB	Female, 19 years; nonoccupational; ingestion (pica), 4-5 moth pellets daily for 2.5 years	Increased skin pigmentation in areas 3-7 cm in diameter on limbs; mental sluggishness; tremor; upon withdrawal, unsteady gait along with decrease in pigmentation	Frank and Cohen, 1961

TABLE 8-3 (cont.)

Chemical/Mixture	Subject and Exposure	Effects	Reference
1,4-DCB	Male, 69 years; nonoccupational; dermal exposure, presumably interrupted; episode precipitated by use of chair treated with 1,4-DCB	Dyspnea followed by stiff neck; "tightness" in chest, "gas pains" in abdomen; symmetrical petechia and purpura on extremities, swelling discomfort; stool occult blood positive, blood cells in urine; and increased BUN; basophil degranul. test positive for 1,4-DCB; diagnosis: allergic (anaphylactoid) purpura and acute glomerulonephritis	Nalbandian and Pierce, 1965
1,4-DCB (and naphthalene)	Female, 68 years; occupational; inhalation and dermal exposure to mothproofing agents for 1 month/year for 39 years	Aplastic anemia	Harden and Baetjer, 1978

*Source: U.S. EPA, 1980c

exposed to airborne concentrations averaging 270-630 mg/m³. Workers complained of eye and nose irritation at levels >800 mg/m³. Another survey, conducted at a 1,2-dichlorobenzene manufacturing facility, reported ambient levels of 6-264 mg/m³ (90 mg/m³ average) (Hollingsworth et al., 1958). Occasional medical examinations, including hematology and urinalysis, revealed no evidence of injury or adverse hematologic effects attributable to the exposure.

8.2.3. Summary. Epidemiologic data are insufficient to evaluate dose-response associations. Possible chronic effects of exposure to the dichlorobenzenes are indicated by case reports of the chronic exposure of individuals, i.e., repeated exposures over a period of more than a year, suggesting a common set of toxic effects, those of the reticuloendothelial and hematopoietic systems and those of the liver. Of the 23 cases in the literature, 17 involved pathological changes in the blood or liver, including chronic lymphoid leukemia, acute hemolytic anemia, aplastic anemia and bone marrow hyperplasia. Although the exposures in these cases are not well defined in time and often involve other toxic substances, together they suggest a common pathologic action of the dichlorobenzenes on bone marrow and other organs of the blood-forming system. The one available epidemiologic study (Zapata-Gayon et al., 1982) supports this generalization in that the reported short-term exposure to 1,2-dichlorobenzene (8 hours/day for 4 days) produced alterations in the chromosomes of leukocytes. This epidemiologic study did not establish an association between chromosomal alterations and the pathologic changes that characterize the case studies.

8.3. MAMMALIAN TOXICOLOGY

8.3.1. Acute Toxicity. Many studies have investigated the acute toxicity of 1,2- and 1,4-dichlorobenzene, but no studies were available on

1,3-dichlorobenzene. In general, the acute toxic effects of 1,2- and 1,4-dichlorobenzene have shown a similar profile of effects in all of the species tested and depend to a certain degree on the route of administration. For oral administration, these effects include, initially, increased lacrimation, salivation and excitation followed by ataxia, dyspnea and death from respiratory paralysis usually within 3 days. On autopsy, the animals were found to have enlarged livers with necrotic areas, submucosal hemorrhages of the stomach, necrotic changes of the kidneys and brain edema. After acute inhalation, the toxic effects observed were eye and nose irritation, liver and kidney necrosis and central nervous system depression. Lethal doses for both oral and inhalation routes for 1,2-dichlorobenzene tend to be one-half to two-thirds of the values for 1,4-dichlorobenzene. Acute dermal application of 1,2-dichlorobenzene results in local irritation and absorption of a lethal amount. Acute toxicity data for 1,2- and 1,4-dichlorobenzene, as compiled by U.S. EPA (1980), are given in Tables 8-4 and 8-5, respectively.

Hollingsworth et al. (1956, 1958) determined the acute oral toxicity of 1,2-dichlorobenzene (50% in olive oil) in 10 guinea pigs of mixed sex and 1,4-dichlorobenzene (20 or 50% in olive oil) in rats and (50% in olive oil) in guinea pigs. The intubation of guinea pigs with 1,2-dichlorobenzene in single oral doses of 800 mg/kg resulted in loss of body weight, but was survived by all subjects, whereas 2000 mg/kg doses were fatal to all subjects. Intubation of rats and guinea pigs with 1,4-dichlorobenzene in single oral doses of 1000 mg/kg and 1600 mg/kg bw, respectively, were survived by all the test animals, while doses of 4000 mg/kg and 2800 mg/kg bw were found to be lethal to rats and guinea pigs, respectively.

TABLE 8-4

Acute Toxicity of 1,2-Dichlorobenzene*

Species	Route of Administration	Concentration or Dose	Regimen	Effects	Reference
Rat	inhalation	5872 mg/m³	7 hours	lethal in 4/5	Hollingsworth et al., 1958
Rat	inhalation	4249 mg/m³	7 hours	lowest lethal concentration	Christenson and Fairchild, 1976
Rat	inhalation	3239 mg/m³	7 hours	eye irritation, CNS depression, liver and kidney damage	Hollingsworth et al., 1958
Guinea pig	inhalation	4808 mg/m³	24 hours	lowest lethal concentration	Christenson and Fairchild, 1976
Guinea pig	oral	2000 mg/kg	single	100% mortality	Hollingsworth et al., 1958
Guinea pig	oral	800 mg/kg	single	weight loss	Hollingsworth et al., 1958
Rabbit	oral	1875 mg/kg	single	LD_{50}	Varshavskaya, 1967a
Rat	oral	2138 mg/kg	single	LD_{50}	Varshavskaya, 1967a
Mouse	oral	2000 mg/kg	single	LD_{50}	Varshavskaya, 1967a
Guinea pig	oral	3375 mg/kg	single	LD_{50}	Varshavskaya, 1967a
Rat	dermal	unspecified daily for 5 applications	twice	lethality	Riedel, 1941
Mouse	intravenous	520 mg/kg	single	lowest lethal concentration	Christenson and Fairchild, 1976
Rabbit	intravenous	330 mg/kg	single	lowest lethal concentration	Christenson and Fairchild, 1976

*Source: U.S. EPA, 1980c

TABLE 8-5

Acute Toxicity of 1,4-Dichlorobenzene*

Species	Route	Concentration or Dose	Regimen	Effects	Reference
Rabbit	inhalation	10^5 mg/m^3	30 minutes daily	CNS depression, eye and nose irritation	Domenjoz, 1946
Rat	inhalation	10^5 mg/m^3	30 minutes daily	CNS depression, eye and nose irritation	Domenjoz, 1946
Guinea pig	inhalation	10^5 mg/m^3	30 minutes daily	irritation, CNS depression, and death	Domenjoz, 1946
Guinea pig	oral	2800 mg/kg	single	100% lethal	Hollingsworth et al., 1958
Guinea pig	oral	1600 mg/kg	single	100% survival	Hollingsworth et al., 1958
Rabbit	oral	2812 mg/kg	single	LD_{50}	Varshavskaya, 1967a
Rat	oral	500 mg/kg	single	LD_{50}	Christenson and Fairchild, 1976
Rat	oral	4000 mg/kg	single	100% lethal	Hollingsworth et al., 1958
Mice	s.c.	5145 mg/kg		LD_{50}	Irie et al., 1973

*Source: Modified from U.S. EPA, 1980c

CNS = Central nervous system; s.c. = subcutaneous

Irie et al. (1973) reported the toxicity of 1,4-dichlorobenzene administered subcutaneously to mice. They reported an LD_{50} of 5.145 g/kg. Inhalation of 1,4-dichlorobenzene (dose not specified) resulted in metachromasia of the nuclei and cytoplasm of liver cells.

The induction of hepatic porphyria by oral administration of dichlorobenzene has been reported in several studies. Rimington and Ziegler (1963) gave rats 1,2- and 1,4-dichlorobenzene at levels that increased over several days to 455 and 770 mg/kg, respectively. Clinical observations of toxicity included anorexia, weakness, clonic contractions, hepatomegaly and liver degeneration and focal necrosis. The metabolic alterations seen were increased urinary excretion of uroporphyrin, porphobilinogen and aminolevulinic acid (1,4-dichlorobenzene only). The authors noted that 1,2-dichlorobenzene appeared more acutely toxic and damaging to the liver, while 1,4-dichlorobenzene was more porphyrogenic. Poland et al. (1971) also induced hepatic porphyria in rats by the daily administration of 800 mg/kg 1,3-dichlorobenzene over a 9-day period. Urinary coproporphyrin excretion increased 1 day after the first dose, peaked at day 3 and then decreased to a level 3 times the pre-dosing concentration. The investigators also found that the administration of 1, 3 or 5 doses of 1,3-dichlorobenzene enhanced the metabolism of hexabarbital and bishydroxycoumarin, and interpreted these results to indicate that 1,3-dichlorobenzene induced drug-metabolizing enzymes and enhanced its own degradation.

Enhancement of xenobiotic metabolism of the liver by the dichlorobenzenes and other halogenated benzenes has been confirmed by other studies. Ariyoshi et al. (1975a) treated female Wistar rats orally for 3 days with 250 mg/kg/day of each of the dichlorobenzene isomers. The activities of several hepatic drug-metabolizing enzymes were increased by these treatments, although none of the isomers increased the liver content of cytochrome P-450. Carlson and Tardiff (1976) also studied the effect of 1,4-dichlorobenzene and other halogenated benzenes on hepatic metabolism. Rats orally administered 10-40 mg/kg of the compound for 14 days showed increased activity of several metabolic enzymes, glucuronyltransferase and the detoxification of hexobarbital and O-ethyl-O-p-nitrophenyl-phenyl-phosphonothioate (EPN).

8.3.2. Subchronic Toxicity. Many subchronic toxicity studies of 1,2- and 1,4-dichlorobenzene have been conducted on the oral and inhalation routes of administration. Although the majority of these studies have been on 1,4-dichlorobenzene, the available data indicate that effects similar to those for 1,4-dichlorobenzene result from exposure to 1,2- and 1,3-dichlorobenzene. In the subchronic inhalation studies (i.e., those using repeated doses over a period of weeks or months), the toxic effects noted at low doses (<1000 mg/m^3 but >600 mg/m^3) were growth depression, increased liver and kidney weight and liver necrosis. At higher inhalation doses (>1000 mg/m^3), the toxic effects were liver, lung and kidney pathology, central nervous system depression, granulocytopenia and death. The lowest level at which no adverse effects were found was 580 mg/m^3 of 1,4-dichlorobenzene administered via inhalation to several species for 7 hours/day, 5 days/week, over a 6- to 7-month period (Hollingsworth et al., 1956). Subchronic toxicity data for 1,2- and 1,4-dichlorobenzene are presented in Tables 8-6 and 8-7, respectively.

Hollingsworth et al. (1958) exposed via inhalation groups of 20 rats, 8 guinea pigs and 2 rabbits of each sex plus 2 female monkeys to repeated exposures of 560 mg/m^3 1,2-dichlorobenzene for 7 hours/day, 5 days/week

TABLE 8-6

Subchronic Toxicity of 1,2-Dichlorobenzene*

Route	Concentration or Dose	Regimen	Subject	Effect	Reference
Inhalation	560 mg/m³	7 hours/day, 5 days/week, 6-7 months	rat, guinea pig, rabbit, monkey	No effect on several parameters except decreased spleen weights in male guinea pigs	Hollingsworth et al., 1958
	290 mg/m³	7 hours/day, 5 days/week 6.5 months	rat, guinea pig	No effect on several parameters	Hollingsworth et al., 1958
	455 mg/m³	daily up to 15 days	rat	Hepatic porphyria	Rimington and Ziegler, 1963
Oral	376 mg/kg (tube)	5 days/week, 138 doses	rat	Liver, kidney weight increase; cloudy swelling in liver.	Hollingsworth et al., 1958
	188 mg/kg (tube)	5 days/week, 138 doses	rat	Increase in liver and kidney weight	Hollingsworth et al., 1958
	18.8 mg/kg (tube)	5 days/week, 138 doses	rat	No effects noted	Hollingsworth et al., 1958
	0.01-0.1 mg/kg/day	5 months	rat	Hematopoietic system; altered conditioned reflexes; increased prothromb time and altered enzyme activities	Varshavskaya, 1967a
	500 mg/kg	5 days/week, 13 weeks	rat	Increased liver weights; polyuria in males; increased urinary porphyrins; hepatic necrosis and degeneration; renal tubular degeneration; thymic lymphoid depletion; and hematologic and clinical changes	NTP, 1982
	250 mg/kg	5 days/week, 13 weeks	rat	Increased liver weights; hematologic and clinical changes; hepatic necrosis	NTP, 1982
	125 mg/kg	5 days/week, 13 weeks	rat	Increased liver weights; hematologic and clinical changes; some hepatic necrosis	NTP, 1982
	60 mg/kg	5 days/week, 13 weeks	rat	Hematologic and clinical changes	NTP, 1982
	30 mg/kg	5 days/week, 13 weeks	rat	Hematologic and clinical changes	NTP, 1982

TABLE 8-6 (cont.)

Route	Concentration or Dose	Regimen	Subject	Effect	Reference
Oral (cont.)	500 mg/kg	5 days/week, 13 weeks	mouse	Increased mortality; increased liver weights; increased urinary and liver porphyrins; hepatic necrosis and degeneration; heart and skeletal muscle lesions; lymphoid depletion of thymus and spleen	NTP, 1982
	250 mg/kg	5 days/week, 13 weeks	mouse	Hepatic necrosis and degeneration in males; no effects in females	NTP, 1982
	30, 60, 125 mg/kg	5 days/week, 13 weeks	mouse	No effects	NTP, 1982
Subcutaneous	unspecified	repeated	rabbit	Blood dyscrasias, (agranulocytosis)	Ware and West, 1977

*Source: Modifed from U.S. EPA, 1980c

TABLE B-7

Subchronic Toxicity of 1,4-Dichlorobenzene*

Route	Concentration or Dose	Regimen	Subject	Effect	Reference
Inhalation	10^5 mg/m³	0.5 hours/day, 5-9 days	rabbit	Granulocytopenia; irritation; CNS and lung toxicity; death (12/18)	Zupko and Edwards, 1949
	4800 mg/m³	8 hours/day, 5 days/week, up to 69 exposures	rat, guinea pig, rabbit	Severe irritation; CNS depression and collapse; liver, kidney, lung pathology; deaths	Hollingsworth et al., 1956
	4600-4800 mg/m³	8 hours/day, 5 days/week,	rabbit	Tremors, weakness, nystagmus; some deaths	Pike, 1944
	2050 mg/m³	7 hours/day, 5 days/week, 6 months	rat, guinea pig	Growth depression, increased liver, kidney weight; liver pathology (necrosis, fatty degeneration, swelling, fibrosis)	Hollingsworth et al., 1956
	1040 mg/m³	7 hours/day, 5 days/week, 16 days	rat, guinea pig	Increased liver, kidney weight (rat); lung, liver pathology	Hollingsworth et al., 1956
	950 mg/m³	7 hours/day, 5 days/week, 157-219 days	rat, guinea pig, rabbit, mouse, monkey	Growth depression (guinea pig); increased liver, kidney weight; histological liver changes (cloudy swelling, granular degeneration) in rats, no adverse effects reported in rabbit, mouse or monkey	Hollingsworth et al., 1956
	900 mg/m³	8 hours/day, 2 weeks	mouse	Respiratory excitation; liver pathology, deaths; at serum concentration of 39 mg/ℓ	Irie et al., 1973
	580 mg/m³	7 hours/day, 5 days/week, 6-7 months	rat, guinea pig, mice, rabbit, monkey	No adverse effects on several parameters	Hollingsworth et al., 1956
Oral	1000 mg/kg per dose (tube)	92 doses in 219 days	rabbit	CNS depression; weight loss; liver degeneration and necrosis; deaths	Hollingsworth et al., 1956
	770 mg/kg/day	up to 5 days	rat	Hepatic porphyria	Rimington and Ziegler, 1963
	500 mg/kg/day (tube)	5 days/week, 20 doses	rat	Hepatic centrolobular necrosis; cloudy swelling, renal tubular epithelium, and casts	Hollingsworth et al., 1956

TABLE 8-7 (cont.)

Route	Concentration or Dose	Regimen	Subject	Effect	Reference
Oral (cont.)	5000 mg/kg diet	up to 35 days	Peking duck	Death in 3/10. Retarded growth	Hollingsworth et al., 1956
	500 mg/kg/day (tube)	5 days/week, 263 doses in 367 days	rabbit	CNS depression; weight loss; liver pathology	Hollingsworth et al., 1956
	376 mg/kg/day	5 days/week, 138 doses in 192 days	rat	Increased liver and kidney weight; liver cirrhosis and focal necrosis	Hollingsworth et al., 1956
	250 mg/kg/day	3 days	rat	Induced liver metabolism enzyme system	Ariyoshi et al., 1975a,b
	188 mg/kg/day	5 days/week, 138 doses in 192 days	rat	Increased liver and kidney weight	Hollingsworth et al., 1956
	20-40 mg/kg/day	2 weeks	rat	Induced liver metabolism enzyme system	Carlson and Tardiff, 1976
	18.8 mg/kg/day	5 days/week, 138 doses in 192 days	rat	No adverse effects detected	Hollingsworth et al., 1956

*Source: U.S. EPA, 1980c

for periods ranging up to 7 months. They reported that this exposure regimen did not result in any adverse effects on any of the animal species tested. Inhalation-exposed groups of 20 rats and 8 guinea pigs of each sex plus 10 female mice to repeated exposures of 290 mg/m³ 1,2-dichlorobenzene for 7 hours/day, 5 days/week, for 6.5 months again resulted in no adverse effects on any of the tested animals.

Several species of laboratory animals were exposed to 1,4-dichlorobenzene vapor at each of five concentrations for 7 hours/day (8 for the highest dose group), 5 days/week (Hollingsworth et al., 1956). Effects in animals (rats, guinea pigs, rabbits) exposed to 4800 mg/m³ for up to 69 exposures included: some deaths (up to 25%); marked tremors, weakness, collapse, eye irritation, and reversible eyeground changes in rabbits, but no lens changes; weight loss, liver degeneration and necrosis, cloudy swelling of renal tubular epithelium (rats); and lung congestion and emphysema (rabbits). Effects in rats and guinea pigs exposed at 2050 mg/m³ for 6 months included: growth depression (male guinea pigs); increased liver and kidney weights (male rats); and liver pathology (cloudy welling, fatty degeneration, focal necrosis, cirrhosis) in some of the animals. Effects in animals exposed for as high as 12 exposure over 16 days at 1040 mg/m³ were: increased liver, spleen and kidney weights (guinea pigs); pulmonary edema, congestion, hemorrhage; hepatic centrolobular congestion and granular degeneration (rats). Effects in animals exposed to 950 mg/m³ for 157-219 days included: growth depression (guinea pigs); increased liver weights (rats, guinea pigs) and increased kidney weights (rats); and centrolobular hepatocellular cloudy swelling or granular degeneration (rats). No adverse effects were observed in rats, guinea pigs, rabbits, mice or a monkey exposed at 580 mg/m³ for 6-7 months.

In the corresponding subchronic oral studies, female rats (10/group, strain not specified) were dosed via stomach tube with 18.8, 188 or 376 mg 1,2-dichlorobenzene/kg/day, 5 days/week, for a total of 138 doses over 192 days (50% 1,2-dichlorobenzene in olive oil) (Hollingsworth et al., 1958). No adverse effects on growth or mortality were observed at any dose level. A dose of 376 mg/kg/day resulted in slightly increased liver and kidney wet weights, a slight decrease in spleen wet weight and slight to moderate cloudy swelling in the liver. Slight increases in liver and kidney wet weights were observed at the intermediate dose and no effects were noted at the lowest dose (18.8 mg/kg/day). Application of 1,2-dichlorobenzene to the eyes of two rabbits resulted in slight to moderate pain and slight conjunctival irritation which cleared completely within 7 days.

1,4-Dichlorobenzene was dissolved in olive oil and given by stomach tube to male adult rats (2/group) at 10, 100 or 500 mg/kg 5 days/week for 4 weeks. Centrolobular hepatic necrosis and marked cloudy swelling of renal tubular epithelium with cast formation occurred in animals given 500 mg/kg. No adverse effects were observed at the lower dose levels (Hollingsworth et al., 1956).

White female rats (10/group) were fed either 18.8, 188 or 376 mg/kg of 1,4-dichlorobenzene in olive oil (emulsified with acacia) by stomach tube 5 days/week for a total of 138 doses in 192 days (Hollingsworth et al., 1956). At the highest dosage level of 376 mg/kg/dose, increased liver and kidney weights, and slight hepatic cirrhosis and focal necrosis were observed. [illegible] adverse effects were noted at the lowest dose level (18.8 mg/kg). No cataracts were observed in these exposures. The same investigators fed rabbits (5/group) with 1,4-dichlorobenzene in olive oil by intubation for up to 92 doses in 219 days at a level of 1000 mg/kg/dose. Another group received a dose level of 500 mg/kg/dose 5 days/week for a total of 263 doses in 367 days. Effects at the higher dose level (1000 mg/kg) included: weight loss, tremors, weakness, hepatic cloudy swelling and a few areas of focal necrosis, and deaths. Similar changes, but no deaths, were noted in rabbits on the lower dose regimen. No cataracts were observed. Peking ducks (10/group) fed 1,4-dichlorobenzene in their diet at 0.5% (5000 mg/kg diet) for 35 days experienced retarded growth and 30% mortality in 28 days, but no cataracts were observed (Hollingsworth et al., 1956).

Varashavskaya (1967) administered 1,2-dichlorobenzene in sunflower oil orally to rats at doses of 0.001, 0.01 and 0.1 mg/kg/day over a 5-month period. At the highest dose level, inhibition of erythropoiesis and bone marrow activity was observed. In addition, at this level, adrenal weight and ascorbic acid content decreased, serum alkaline phosphatase and transaminase activity increased, and serum glutathione decreased. Similar effects were noted in the intermediate dose level animals, but not at the lowest dose. These results are in distinct contrast to those of Hollingsworth et al. (1956) who found no effects at a dose level of 18.8 mg/kg after a 6-month administration period.

Subchronic toxicity studies on 1,2-dichlorobenzene were conducted under the auspices of the National Toxicology Program (NTP, 1982). The investigations were conducted using 10 male and 10 female $B6C3F_1$ mice and 10 male and 10 female F344/N rats. The 1,2-dichlorobenzene was administered by gavage using a corn oil vehicle, 5 mℓ/kg, 5 days/week for 13 weeks. The 1,2-dichlorobenzene doses used were 0, 30, 60, 125, 250 or 500 mg/kg.

The 1,2-dichlorobenzene mouse study resulted in a decreased survival rate in the male 250 mg/kg dose group with a mortality rate of 10% (1/10) and in the male and female 500 mg/kg dose groups with mortality rates of 40%

(4/10) and 30% (3/10), respectively (NTP, 1982). Liver weight/body weight ratios were significantly increased in both males and females at 500 mg/kg. Spleen weight/body weight ratios were decreased in all 1,2-dichlorobenzene dosed female groups and uterus weight/body weight ratios were decreased in the 500 mg/kg female group. No biologically significant changes were found during the hematological evaluations. Female mice receiving 500 mg/kg 1,2-dichlorobenzene were found to excrete 6 times more coproporphyrins in their urine and had a 2-fold increase in liver porphyrin concentrations when compared with control mice. No histological effects were observed in the 0, 30, 60 or 125 mg/kg dose groups. The 250 mg/kg dose male mice group was found to have hepatocellular necrosis and degeneration while the females receiving this dose were found to be unaffected. Ninety percent of both the male and female 500 mg/kg dose groups were observed with centrilobular necrosis, necrosis of individual hepatocytes or hepatocellular degeneration. The hearts of the 500 mg/kg dosed animals had mineralization of the myocardial fibers (multiple foci) and the skeletal muscles were observed with some necrosis, myositis and mineralization. Both the male and female 500 mg/kg groups were observed with lymphoid depletion of the thymus and spleen and a yellow-green pigmentation (considered to be hemosiderin) in some of their livers. Based on these results NOELs are determined to be 125 mg/kg for male mice and 250 mg/kg for female mice.

The 1,2-dichlorobenzene rat study resulted in a dose-dependent depression in mean body weight gains over the 13-week period (NTP, 1982). A dose-related increase in liver weights was also observed in both sexes with significant increases in liver weight/body weight ratios in the 125, 250 and 500 mg/kg male and female dose groups. Decreases in spleen and thymus weights and organ weight/body weight ratios were observed in the male 500 mg/kg group. Minimal changes in hematologic parameters were observed in the 500 mg/kg dose groups. An increased number of platelets were found in female rats receiving 60, 125 and 500 mg/kg doses of 1,2-dichlorobenzene. A dose-related increase in serum cholesterol levels were found in males receiving 30, 125, 250 and 500 mg/kg and in females receiving 125-500 mg/kg. A decrease in serum triglycerides was observed at 500 mg/kg (males) and 250 mg/kg (females), and a dose-related increase in total serum protein was observed at 250-500 mg/kg (males) and at 30-500 mg/kg (females). Female rats were observed with minimal increases in serum glucose levels at 1,2-dichlorobenzene doses of 30, 125, 250 and 500 mg/kg. Polyuria was observed in males receiving the 500 mg/kg dose. A 3- to 5-fold increase in urinary uroporphyrins and coproporphyrins were seen in males and females at 500 mg/kg. The liver porphyrin levels were not altered by 1,2-dichlorobenzene at any dose level. Hepatocellular necrosis and focal hepatic necrosis were observed in some of the rats at the 125 mg/kg dose. More hepatocellular necrosis was seen in both males and females at 250 mg/kg. Most of the rats in the 500 mg/kg dose groups had liver lesions, either centrilobular degeneration or hepatic necrosis. The 500 mg/kg male group also had renal tubular degeneration and thymic lymphoid depletion. A yellow-green to gold pigment (believed to be hemosiderin) was also observed in the livers of rats at 250 and 500 mg/kg. Based on these results, a LOAEL for 1,2-dichlorobenzene in rats is determined to be 30 mg/kg.

The effect of subchronic treatment with 1,4-dichlorobenzene has been extensively investigated in guinea pigs (Salamone and Coppola, 1960; Totaro, 1961; Coppola et al., 1963; Totaro and Licari, 1964). Intramuscular injections of 125 mg 1,4-dichlorobenzene (50% in almond oil) daily for 20 days were found to produce weight loss (5-10%), increased blood serum transaminase and increased clotting times.

8.3.3. Chronic Toxicity. Two-year chronic bioassay studies using 1,2-dichlorobenzene were conducted under the auspices of the National Toxicology Program (NTP, 1982). The investigations were conducted using 50 male and 50 female B6C3F1 mice and 50 male and 50 female F344/N rats. 1,2-Dichlorobenzene was administered by gavage in a corn oil vehicle, 5 mℓ/kg, 5 days/week for 103 weeks. The dosage groups used were 0 (vehicle control), 60 and 120 mg/kg.

The 1,2-dichlorobenzene mouse study resulted in a 105-week (exposure duration 103 weeks) survival rate of 52% (26/50), 64% (32/50) and 70% (35/50) in male mice and 66% (33/50), 80% (40/50) and 76% (38/50) in female mice for the 0, 60 and 120 mg/kg dose groups, respectively (NTP, 1982). Mean body weights were comparable between 1,2-dichlorobenzene dosed and control male mice but the female dosed mice were slightly higher in body weight than controls. Histological findings of neoplasms in dosed and control groups will be discussed in Section 8.3.5. Carcinogenicity. No apparent increase in non-neoplastic lesions in the liver, kidney, bone marrow, spleen or other organs of male and female mice were observed as a result of administration of 1,2-dichlorobenzene over the 105-week study period.

The 1,2-dichlorobenzene rat study resulted in 104-105 week (exposure duration 103 weeks) survival rates of 84% (42/50), 72% (36/50) and 38% (19/50) [significantly different from the 60 mg/kg group ($p=0.014$) and 0 mg/kg group ($p<0.001$)] in male rats and 62% (31/50), 66% (33/50) and 64% (32/50) in female rats for the 0, 60 and 120 mg/kg dose groups, respectively (NTP, 1982). Slightly lower mean body weights were observed in the male 120 mg/kg group when compared with the 0 and 60 mg/kg male groups. This was contrasted by higher mean body weights in the female animals in the 120 mg/kg group when compared with the female controls. Histological findings of neoplasms in dosed and control groups will be discussed in Section 8.3.5. Carcinogenicity. No apparent increase in non neoplastic lesions in the liver, kidney, bone marrow, spleen, thymus or other organs of male and female rats were observed as a result of administration of 1,2-dichlorobenzene over the 105-week study period.

8.3.4. Mutagenicity. The capability of the dichlorobenzenes to induce mutations or other alterations of genetic structure has not been extensively investigated, although a recent study (Zapata-Gayon et al., 1982) indicates such research is warranted. As cited in Section 8.2., a higher incidence of chromosomal breaks was observed in the leukocytes of humans accidentally exposed for a short period of time to 1,2-dichlorobenzene vapors (Zapata-Gayon et al., 1982).

Anderson et al. (1972) reported that 1,2-dichlorobenzene did not induce point mutations when tested in *Salmonella typhimurium* (8 unspecified strains) without activation. No conclusions can be drawn from this study because of the lack of details provided and because metabolic activation was not used. In an abstract, Lawlor et al. (1979) evaluated the ability of chlorinated phenols, benzenes and hexanes to induce mutations or DNA damage in bacteria. Tests of 1,2- and 1,4-dichlorobenzene (doses not specified) were negative in five strains of *Salmonella* (TA98, TA100, TA1535, TA1537 and TA1538) with and without rat liver microsomal activation. DNA repair tests with two *Salmonella* and two *E. coli* strains with and without activation indicated the ability of two unspecified chlorobenzenes to cause preferential killing of the DNA repair deficient strains. Because these results were reported in an abstract with insufficient experimental detail, the results cannot be critically evaluated. These negative findings in bacteria were supported by studies conducted for the National Toxicology Program (Appendix M of NTP, 1982). In these studies, 1,2-dichlorobenzene was negative in four Salmonella strains (TA98, TA100, TA1535 and TA1537) when tested with and without metabolic activation at doses as high as 1300 µg/plate.

Prasad and Pramer (1968) reported testing all three isomers of dichlorobenzene in an auxotrophic strain of Aspergillus nidulans, a soil mold. All three compounds increased the frequency of back mutations in the following descending order: 1,4-, 1,3- and 1,2-dichlorobenzene. Abnormal numbers of chromosomes and abnormally shaped nuclei were observed in the root cells of Allium exposed for 4 hours to 1,4-dichlorobenzene vapors (Carey and McDonough, 1943). Sharma and Bhattacharyya (1956) reported chromosomal breakage and nondisjunction in the root tips and flower buds of Nothoscordum fragans, which were treated with saturated aqueous solutions of 1,4-dichlorobenzene. Various mitotic abnormalities were also found in the somatic cells and chromosomes of the root tips of several plant species treated with 1,4-dichlorobenzene (Srivastava, 1966). The aberrations included shortening and thickening of chromosomes, early separation of chromatids, tetraploid cells, binucleate cells, chromosome bridges and chromosome breaks in the heterochromatic regions. Sarbhoy (1980) exposed germinating root tips of Lens esculenta to 1,4-dichlorobenzene vapors and also observed chromosome fragmentation, condensation and bridges and polyploid cells.

8.3.5. Carcinogenicity. The National Toxicology Program (NTP) conducted a 2-year study on 1,2-dichlorobenzene with F344/N rats and with $B6C3F_1$ mice (NTP, 1983). There were 50 animals of each sex for each dosage group in both the rat and mouse studies. The 1,2-dichlorobenzene was prepared in corn oil and administered by gavage 5 days/week for 103 weeks. The dosages used were 0, 60 and 120 mg/kg for each day of dosing. The 1,2-dichlorobenzene was >99% pure with the major impurity found to be 0.84% v/v of 1,4-dichlorobenzene. The stability of the 1,2-dichlorobenzene preparation was monitored.

8.3.5.1. RATS -- In male rats survival was reported (NTP, 1983a) to be significantly reduced in the 120 mg/kg group. The NTP peer review committee for this draft document (NTP, 1983a) suggested that the reduced mortality of this group of animals provides evidence that the MTD had been reached. However, this group had 12 animals which died before the end of the study with gavage solution in the lungs and another 5 that were killed as a result of accident in the gavage procedure. If these 17 accidentally injured or killed animals had survived there would have been comparable survival in this test group to the low dose and control groups. The mortality of the males in this group is, therefore, a function of technical problems with the gavage technique and does not reflect a compound related response. Therefore, the conclusion can be drawn that the reduced survival of these animals does not provide evidence that the maximum tolerated dose had been reached. Body weights of rats in the treated groups were within 1% of those of the controls among males and 12 and 11% higher than controls for female rats in the low and high dose groups respectively after 99 weeks.

The results of the histopathological analysis showed that non-neoplastic lesions were not significantly increased in the treated rats nor were neoplasms other than adrenal pheochromocytoma. The latter (Table 8-8) was significantly increased in the low dose group when compared to controls by the life table test but not in the other statistical tests. The terminal

TABLE 8-8

NTP Bioassay of 1,2-Dichlorobenzene
Analysis of Primary Tumors in Male Rats: Adrenal Pheochromocytomas*

	Vehicle Control	60 mg/kg	120 mg/kg
Tumor Rates			
Overall	9/50 (18%)	16/50 (32%)	6/49 (12%)
Adjusted	20.9%	40.5%	21.7%
Terminal	8/42 (19%)	13/36 (36%)	2/18 (11%)
Statistical Tests			
Life Table	p = 0.201	p = 0.039	p = 0.380
Incidental Tumor Test	p = 0.499 N	p = 0.070	p = 0.420 N
Cochran-Armitage Trend, Fisher Exact Tests	p = 0.285 N	p = 0.083	p = 0.303 N

*Source: NTP, 1982

incidence of adrenal pheochromocytoma in males was 36% (13/36) in the low dose group compared with 19% (8/42) in controls. The historical incidence of adrenal tumors in male F344/N rats receiving corn oil by gavage, based on data from seven different laboratories is 153/986 (15.5%).

In rats, therefore, under conditions of this test, carcinogenicity was not in evidence. However, based on the following observations from the 2-year study, a larger dose probably could have been tolerated:

1) there was no increase in mortality in treated groups when compared with controls,
2) there was no loss of weight in the treated groups compared to the controls,
3) there was no evidence of life-threatening pathology in the treated groups compared to the controls.

The question is then raised as to whether a higher dose could, or should, have been tested. In the 13-week dose selection study rats were given doses of 60, 125, 250, 500 and 1000 mg/kg on each day of dosing and no deaths occurred at any dose up to and including 500 mg/kg in both male and female rats. Weight gain was within 7% of the controls in female rats through the 500 mg/kg group and through the 250 mg/kg group in male rats. Mortality and weight decrement have been traditionally used as indicators of levels of toxicity which would be expected to reduce the number of animals at risk in a long-term study and, hence, indicate when inappropriately high doses were being used. Use of these criteria indicates that a dose over 120 mg/kg could have been used in the 2-year study.

Another consideration in the design of this study is that of the pharmacokinetics of 1,2-dichlorobenzene. Azouz et al. (1955) studied the metabolism and excretion of both 1,2-dichlorobenzene and 1,4-dichlorobenzene in rabbits given 500 mg/kg. They found that 1,2-dichlorobenzene took 5-6 days for complete metabolism and elimination. Kimura et al. (1979) found similar results with oral administration of 200-800 mg/kg 1,4-dichlorobenzene in Wistar rats. A regimen which gives 120 mg/kg for only 5 days, followed by no treatment for 2 days, may not be providing effective continuous exposure, an element important in the chronic toxicity testing rationale.

In summary, the assay of 1,2-dichlorobenzene in F344 rats did not give evidence of carcinogenicity. However higher doses probably could have been tolerated and the assay was not as sensitive as it could have been.

8.3.5.2. MICE -- In $B6C3F_1$ mice 500 mg/kg for each day given in the 13-week study resulted in increased mortality and significant weight decrement (47% in males and 67% in females). Only 1 male animal died at the 250 mg/kg dose at 13 weeks. Histopathology of the liver, myocardium, skeletal muscle, thymus and spleen were found at 500 mg/kg but the only compound-related lesion at the 250 mg/kg dose was found in males; necrosis of individual hepatocytes (2/10), hepatocellular degeneration (1/10) and pigment deposition (1/10). No compound related lesions were found in females at this dose. At the 250 mg/kg dose, the body weights did not different significantly from controls in this 13-week study. Some liver pathology was observed in male mice (4/10) that received a dose of 250 mg/kg in this 13-week study. The question of whether this level of pathology, in a potential target organ, is sufficient justification for selecting 120 mg/kg as the highest dose in the 2-year study must be raised. As in the chronic study with rats there were no significant weight or survival differences between treated and control groups.

After histopathological analysis the only non-neoplastic lesion which appeared to be treatment-related was a dose-related trend in tubular regeneration of the kidney in male mice. Increases in non-neoplastic lesions of the liver were not found. In fact there was a dose related decrease in hepatocellular adenoma incidence and a significant decrease in this tumor when the highest dose group and controls were compared. It should be noted that male $B6C3F_1$ mice had an incidence of 38% (19/50) of liver adenoma or carcinoma in the control group. The problem of a high background incidence of this type in mice is repeatedly at issue in terms of suitability of these animals for testing when liver tumors are the potential target lesions.

In conclusion, neither the rat nor the mouse study gave evidence of carcinogenicity under the test conditions, but the doses selected were probably below the MTD in both species, reducing the sensitivity of the assays. The marginal increase in adrenal pheochromocytoma in rats should be noted as this lesion appears with hexachlorobenzene, also at a relatively low dose. If the IARC criteria for classifying carcinogens were used, this evidence is clearly inadequate for developing any conclusions concerning the carcinogenicity of 1,2-dichlorobenzene in humans.

8.3.6. **Reproductive and Teratogenic Toxicity.** No data on the reproductive and teratogenic toxicity of the dichlorobenzenes were available for review; however, dichlorobenzenes have been demonstrated to cross the placenta (Dowty and Lasiter, 1976).

8.4. INTERACTIONS

As indicated in Section 8.2., halogenated benzenes, including the dichlorobenzenes, have the ability to induce hepatic xenobiotic metabolizing enzymes (Ariyoshi et al., 1975a,b; Carlson and Tardiff, 1976; Carlson, 1977). This type of induction theoretically will alter the metabolism of other compounds; thus, the toxicity resulting from the concurrent exposure to the dichlorobenzenes and other compounds may be different from the exposure to the individual chemicals. One study was available that investigated the effect of dichlorobenzene on the toxicity of other compounds (Townsend and Carlson, 1981). Mice were orally administered 0.1 mmol/kg (18 mg/kg bw) of 1,4-dichlorobenzene and other chlorinated and brominated benzenes daily for 7 days, after which the mice were used in the determination of LD_{50} values for four organophosphorus insecticides. The treatment with 1,4-dichlorobenzene was found to decrease the lethality of parathion and paraoxon by ~50%, although other compounds were much more effective. In addition, Carlson and Tardiff (1976) observed that administration of 1,4-dichlorobenzene (10-40 mg/kg for 14 days to rats) enhanced the detoxification of hexobarbital and EPN.

Harden and Baetjer (1978) reported a human case of aplastic anemia following exposure to 1,4-dichlorobenzene and naphthalene. While a single case report cannot be considered convincing evidence for an interactive effect, the possibility of interactions cannot be dismissed.

8.5. SUMMARY

The available data on the pharmacokinetics of the dichlorobenzenes indicate that these compounds are absorbed through the lungs, skin and gastrointestinal tract and rapidly distributed to many tissues, especially those with a high lipid content. Metabolism is accomplished by oxidation to dichlorophenols which are conjugated as glucuronides and sulfates. Elimination, primarily through the urine, appears to be rapid, although the data are insufficient to make quantitative estimates of the rate. Biliary excretion does occur but little of the biliary excreted dichlorobenzene has been found in the feces, probably due to enterohepatic recirculation. The dichlorobenzenes, as well as the other chlorinated benzenes, are capable of bioaccumulation (see Section 5.3.).

Data on possible effects in humans were available in a number of case reports and in a single epidemiologic study. The case studies demonstrate the ability of the dichlorobenzenes to be absorbed through the lungs and gut and their acute and subchronic toxicity. Many of these reports, in which exposure may have occurred over several years, noted toxic effects in the blood, such as chronic lymphoid leukemia and anemia, as well as effects on the liver. The one available occupational study reported chromosomal alterations in leukocytes resulting from a short-term exposure to 1,4-dichlorobenzene. Taken together, these studies suggest a possible toxic action of dichlorobenzenes on bone marrow and other organs of the blood-forming system.

Studies of the acute and subchronic toxicity of the dichlorobenzene isomers indicate that, in general, these compounds have similar target organs and effects. At oral doses ranging from 125-1000 mg/kg over periods of up to 6 months, the dichlorobenzenes cause central nervous system depression, injury to liver, kidney, heart, thymus and spleen, and hepatic and urinary porphyria; however, one study reported that a low dose of 0.01 mg/kg over a 5-month period inhibited erythropoiesis and bone marrow activity. The subchronic oral toxicity studies in rats provide two estimates of NOEL values: 0.001 mg/kg (Varashavskaya, 1967) for 1,4-dichlorobenzene and 18.8 mg/kg for 1,2- and for 1,4-dichlorobenzene (Hollingsworth et al., 1956, 1958). The NTP (1982) subchronic oral study on 1,2-dichlorobenzene in mice provided higher estimated NOEL values of 125 and 250 mg/kg for males and females, respectively. A 2-year NTP chronic oral gavage study on 1,2-dichlorobenzene in rats and mice, conducted primarily as a carcinogenesis bioassay at the 60 and 120 mg/kg dose levels, resulted in only increased mortality in the male rats given 120 mg/kg. Acute and subchronic inhalation studies of dichlorobenzenes indicate similar toxic effects and target organs as seen in the oral studies. The effects occurred at doses $\geq$950 mg/m^3; inhalation NOELs were reported as 580 mg/m^3 for 1,2-dichlorobenzene (Hollingsworth et al., 1956) and 290 mg/m^3 for 1,4-dichlorobenzene (Hollingsworth et al., 1958).

The mutagenicity studies with bacteria were lacking in experimental detail, but suggest that the dichlorobenzenes are probably not mutagenic in bacteria. However, several studies with mold and plant cultures treated with dichlorobenzenes have reported mutations and chromosomal aberrations. Because chromosomal aberrations were also observed in human workers exposed to 1,2-dichlorobenzene, the weight of available evidence suggests that the dichlorobenzenes are clastogens. The carcinogenic activity of one isomer, 1,2-dichlorobenzene, was tested in the NTP bioassay program in two rodent species at doses of 60 and 120 mg/kg. No evidence of carcinogenic activity was found under the test conditions.

9. TRICHLOROBENZENES

The trichlorobenzenes are produced in relatively small amounts (1.3-3.95 million kg/year is the estimated 1983 production) (U.S. EPA, 1983) and are used primarily as chemical intermediates, solvents, insecticides, and coolants and insulators in electrical equipment (Hawley, 1977; Slimak et al., 1980). Trichlorobenzenes have been detected in all environmental media including drinking water (see Section 4.3.), and have been found to bioaccumulate in fish (see Section 5.3.). In addition to the exposure of humans during the manufacture and use of trichlorobenzenes, exposure is likely to result from inhalation and ingestion of contaminated air and water.

9.1. PHARMACOKINETICS

9.1.1. Absorption.

No quantitative studies on the absorption of the trichlorobenzenes from the gastrointestinal tract, skin or lungs were found. Information on absorption may be obtained from data describing elimination. Male Charles River rats (16 in the group) excreted a mean of 84%, and two female rhesus monkeys excreted a mean of 40% of the orally (by gavage) administered dose of 10 mg ^{14}C-1,2,4-trichlorobenzene/kg in the 24-hour urine, while fecal elimination accounted for only 11 and 1%, respectively (Lingg et al., 1982). The results indicate that in these species, this isomer is well absorbed through the gastrointestinal tract. Two Chinchilla female rabbits given doses of 500 mg 1,3,5-trichlorobenzene/kg in arachis oil by gavage expired ~10% of the administered dose from the lungs in 9 days (Parke and Williams, 1960). These investigators also observed elimination of urinary and fecal metabolites, but quantities or percentages were not reported.

That the trichlorobenzenes are absorbed by the respiratory tract and by the skin can be inferred from toxicity studies using the inhalation (Kociba et al., 1981) and dermal (Brown et al., 1969) routes of exposure. These studies, however, were not designed to give information on rates of absorption.

9.1.2. Distribution. Smith and Carlson (1980) examined the distribution of ^{14}C-1,2,4-trichlorobenzene in groups of four male Sprague-Dawley rats on days 1, 6, 11 and 16 after oral daily dosing with 181.5 mg/kg (1 mmol/kg) for 7 days. Their data indicate that the adrenals initially had the highest concentration of radiolabel. This level declined rapidly; however, by day 11 it was less than twice the background of the other tissues. Abdominal fat had the highest concentration at the end of 1 day (Table 9-1) and maintained detectable concentrations (20% of the day 1 level) for the duration of the observation period (16 days). The liver also maintained detectable levels throughout the recovery period, retaining ~30% of the day 1 level by day 16. These authors also found that starvation for 4 days had no observed effect on the distribution of ^{14}C-trichlorobenzene in fat or liver.

Parke and Williams (1960) reported the distribution of 1,3,5-trichlorobenzene in one rabbit on day 8 following oral administration of 500 mg/kg as follows: 13% of the administered dose was detected in the feces, 23% (4% as monochlorobenzene) in the gut, 5% in the pelt, 5% in depot fat (exclusive of pelt) and 22% in the carcass.

9.1.3. **Metabolism.** No metabolic studies following the inhalation of trichlorobenzenes were available for review, but the metabolic fate following oral and/or intravenous (i.v.) or intraperitoneal (i.p.) administration has been characterized in rabbits (Jondorf et al., 1955; Parke and Williams, 1960; Kohli et al., 1976) and in rats and monkeys (Lingg et al., 1982).

Jondorf et al. (1955), using spectrophotometric analysis, studied the metabolism of all three isomers of trichlorobenzene in groups of 3 or 4

TABLE 9-1

Distribution of ^{14}C-Labeled 1,2,4-Trichlorobenzene in Rat Tissues after Oral Dosing with 181.5 mg/kg/day for 7 Days[a]

Tissue	Activity (dpm/g tissue)[b]			
	Day 1	Day 6	Day 11	Day 16
Abdominal fat	2033±439	642±54	342±10	408±39
Liver	1075±87	442±22	308±21	317±18
Adrenals[c]	754±132	246±22	d/	
Muscle	400±30	d/		
Kidney	1471±167	404±43	d/	
Heart	438±14	d/		
Spleen	404±14	d/		

[a] Source: Smith and Carlson, 1980

[b] Each value is the mean ± SE for 4 rats, except for abdominal fat on day 1, which was for three rats.

[c] Total for both adrenals; they were not weighed.

[d] Value less than twice background; further analyses were not performed.

Chinchilla rabbits given oral doses of 500 mg/kg. The results indicated that the 1,2,3- isomer was metabolized to 2,3,4-trichlorophenol (TCP), to 3,4,5-TCP to a lesser degree, and to small amounts of 3,4,5-trichlorocatechol. During the 5 days after administration, 50% of the dose was excreted in the urine as glucuronic acid conjugates, 12% as sulfuric acid (sulfate) conjugates and 0.3% as 2,3,4-trichlorophenylmercapturic acid. The 5-day urinary metabolites of 1,2,4-trichlorobenzene were represented by glucuronide conjugates (27%), sulfuric acid conjugates (11%) and 2,3,5- and 2,4,5-trichlorophenylmercapturic acid (0.3%). The major phenols formed were 2,4,5- and 2,3,5-TCP. For the 1,3,5- isomer, 20% was excreted as glucuronide and 3% as sulfuric acid conjugates. No mercapturic acid was found, 2,4,6-trichlorophenol was the only phenol detected in the urine, and some unchanged 1,3,5-trichlorobenzene was present in the feces. To further characterize and clarify the metabolic fate of the 1,3,5- isomer, Parke and Williams (1960) followed the 9-day urinary excretion in 2 or 3 female Chinchilla rabbits treated orally with 500 mg of the isomer/kg. For the first 3 days, the rabbits eliminated 2,4,6-TCP along with some minor monochlorophenols, while from day 4 to 9, 4-chlorophenol was detected more prominently along with 2,4,6-TCP and ~1% of the dose as 4-chlorocatechol.

Using GC-MS analysis, Kohli et al. (1976) examined the metabolism of the three trichlorobenzene isomers following i.p. injection of 60-75 mg/kg doses in vegetable oil to male rabbits (number and strain not reported). In agreement with the results of Jondorf et al. (1955), the major urinary metabolites of 1,2,4-trichlorobenzene were 2,4,5- and 2,3,5-TCP. The major metabolite of 1,2,3-trichlorobenzene was 2,3,4-TCP, with 2,3,6- and 3,4,5-TCP as minor urinary metabolites. The 1,3,5- isomer was metabolized to

2,3,5- and 2,4,6-TCP and a third, more polar metabolite, was tentatively identified as a dichlorobenzene with 2 hydroxyl and 1 methoxyl substituents.

Lingg et al. (1982) investigated the metabolism of 1,2,4-trichlorobenzene in groups of 16 male Charles River rats and groups of 2 female rhesus monkeys following oral or i.v. administration of 10 mg/kg doses and found similar phenolic metabolites to those observed in the rabbit. These researchers were also able to characterize some species specific conjugates. An isomeric pair of 3,4,6-trichloro-3,5-cyclohexadiene-1,2-diol glucuronides accounted for 48-61% of the 24-hour urinary metabolites in the monkeys. Also found were glucuronides of 2,4,5- and 2,3,5-TCP and unconjugated TCP, which accounted for 14-37 and 1-37% of the urinary metabolites, respectively. In the rat, the 2,4,5- and 2,3,5- isomers of N-acetyl-S-(trichlorophenyl)-L-cysteine accounted for 60-62% of the urinary metabolites. Minor urinary metabolites included 2,4,5- and 2,3,5-trichlorothiophenol and free 2,3,5- and 2,3,4-TCP, which accounted for 28-33 and 1-10% of the material excreted, respectively.

On the basis of the studies of Lingg et al. (1982) and Kohli et al. (1976), it is apparent that there may be differences among species in the metabolism of 1,2,4-trichlorobenzene. It seems likely that these differences will extend to the other isomers of trichlorobenzene as well. Both reports postulated the same first step in metabolism (i.e., initial formation of arene oxide intermediates), but indicated differences in the subsequent metabolic reactions. In the rat, conjugation of the intermediate with glutathione was postulated to account for the sulfur-containing urinary metabolites. In the monkey, hydrolysis of the arene oxide to the dihydrodiol and the absence of sulfur-containing metabolites seemed to preclude the involvement of glutathione (Lingg et al., 1982). As proposed by Kohli et al. (1976) and illustrated in Figure 9-1, formation of the isomeric trichlorophenols from the arene oxide intermediates can proceed either by direct opening of the C-O bond or by the NIH shift of chlorine.

Differences in the rate of metabolism of the different isomers within a species have been attributed to the positions of the chlorine atoms on the benzene ring, with the presence of two adjacent unsubstituted carbon atoms facilitating the formation of the arene oxide intermediate. Halogenated benzenes without adjacent unsubstituted carbons may still be metabolized via an arene oxide intermediate but at a reduced rate, and should show evidence of a NIH shift (Matthews and Kato, 1979).

9.1.4. Excretion. Lingg et al. (1982) measured the 24-hour excretion of radioactivity in the urine and feces of 16 male Charles River rats and 2 rhesus monkeys given a single 10 mg/kg i.v. or oral dose of ^{14}C-1,2,4-trichlorobenzene. In the rat, 84% of the oral dose and 78% of the i.v. dose were excreted in the urine by 24 hours; 11 and 7%, respectively, were the amounts identified in the feces. In the monkeys, 40% of the oral dose and 22% of the injected dose appeared in the urine and <1% in the feces. Smith and Carlson (1980) orally administered 181.5 mg/kg/day (1 mmol/kg/day) of ^{14}C-1,2,4-trichlorobenzene in corn oil to 4 Sprague-Dawley rats for 7 days and followed the excretion of radioactivity in the feces and in the urine during administration and up to 21 days after the first dose. Fecal elimination rose slightly during the first 3 days of dosing, after which it declined rapidly and was essentially complete at 15 days of collection, accounting for ~4% of the total dose. Urinary excretion followed a similar pattern; however, at 21 days after the first dose, radioactivity was still detectable. Total urinary excretion to this time accounted for ~72% of the total administered radioactivity. As noted by Lingg et al. (1982), the

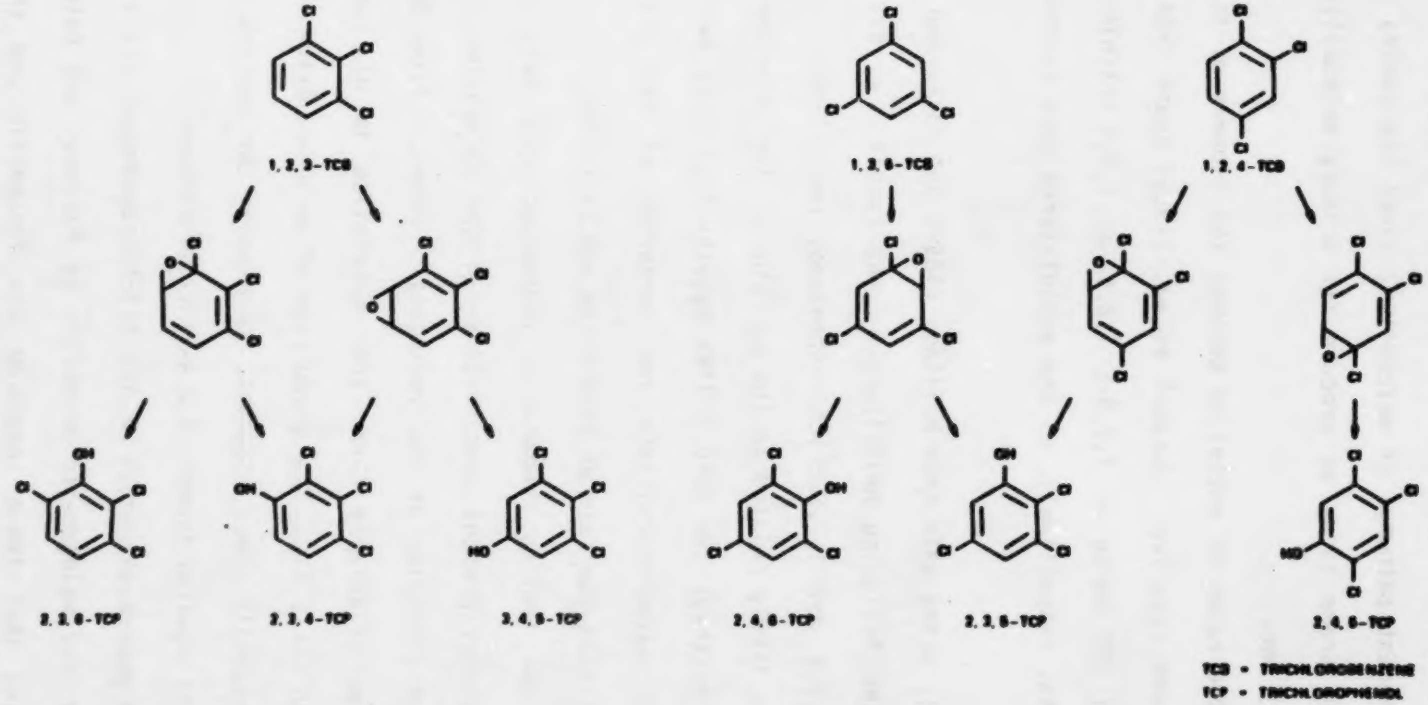
1,2,3-TCB
1,3,5-TCB
1,2,4-TCB
Cl
OH
HO
O
2,3,6-TCP
2,3,4-TCP
3,4,5-TCP
2,4,6-TCP
2,3,5-TCP
2,4,5-TCP
TCB = TRICHLOROBENZENE
TCP = TRICHLOROPHENOL

FIGURE 9-1

Metabolic Pathways for Trichlorobenzene (TCB) Isomers Through Arene Oxide Intermediates in Rabbits

Source: Adapted from Kohli et al., 1976

differences in the excretion rate between the rat and monkey may be attributable to their different pathways of metabolism, since the monkey required two steps beyond the arene oxide to produce its urinary metabolite, while the rat required only one.

Differences in the rates of excretion between the isomers of trichlorobenzene have also been reported. Jondorf et al. (1955) found that rabbits given oral doses of 500 mg/kg of 1,2,3-, 1,2,4- or 1,3,5-trichlorobenzene excreted 78, 42 or 9%, respectively, of the administered dose as monophenols in the 5-day urine.

U.S. EPA (1980b), using data from Williams (1959) and Parke and Williams (1960), estimated the following half-lives in the rabbit: 2, 5.5 and 8.5 days for 1,2,3-, 1,2,4- and 1,3,5-trichlorobenzene, respectively. The rate of excretion is most likely related to the position of the chlorine atoms on the benzene ring. Matthews and Kato (1979) hypothesized that two adjacent unsubstituted carbon atoms facilitate the formation of the arene oxide intermediate and increase the rate of metabolism and excretion.

9.1.5. **Summary.** The limited comparative pharmacokinetic data available on the trichlorobenzenes prevent specification of the absorption, distribution, metabolism and excretion of the individual isomers. From the available data, it seems relatively clear that metabolism in at least three species has a common first step, the production of an arene oxide intermediate. Subsequent metabolic steps, however, vary among the species examined, at least for the most studied isomer, 1,2,4-trichlorobenzene.

In general, the pharmacokinetics of the trichlorobenzenes are similar to those described for the halogenated aromatics by Matthews and Kato (1979). The authors observed that these compounds are lipophilic and that their metabolism and excretion depends on their conversion to polar intermediates. In addition, their lipophilic character provides for ready absorption from the gastrointestinal tract and initial distribution to the more highly perfused tissues, particularly the liver, after which they are either metabolized and excreted or redistributed to adipose tissue or skin. Additional experiments are needed to clarify the relationship of these studies to the metabolism of trichlorobenzenes in humans.

9.2. EFFECTS IN HUMANS

Information on the health effects of trichlorobenzenes in humans is limited to case reports. Rowe (1975) found that an individual exposed to 1,2,4-trichlorobenzene at 3-5 ppm had eye and respiratory irritation. Girard et al. (1969) reported two cases, one in which a 68-year-old woman who often soaked her husband's work clothes in trichlorobenzene developed aplastic anemia, and the other in which a 60-year-old man, who had been occupationally exposed to DDT as well as to mono-, di- and trichlorobenzenes for over 30 years, developed anemia.

9.3. MAMMALIAN TOXICOLOGY

9.3.1. Acute Toxicity. Studies of the acute toxicity of the trichlorobenzenes have been performed in several species using various routes of administration.

Information on the effects of acute inhalation exposure to trichlorobenzenes is limited. In an abstract of a study from the Russian literature (Gurfein and Pavlova, 1960), a single high inhalation dose (doses of 0.005-0.01 mg/ℓ in air or 5-10 mg/m^3 were used) of an unspecified isomer of trichlorobenzene to rats resulted in immediate nervousness and pinkness of mouth, ears and paws. These effects were followed by convulsions and death within 30 minutes, with edema of livers and kidneys observed upon necropsy. Unpublished results of a study performed by Treon (1950) were reported by Coate et al. (1977) and indicated that the target organs of non-lethal acute inhalation exposure to trichlorobenzenes (a weight-to-weight mixture of 8% 1,2,3- and 92% 1,2,4-trichlorobenzene) in cats, dogs, rats, rabbits and guinea pigs included the liver, ganglion cells at all levels of the brain and mucous membranes. Lethal doses resulted in local irritation of the lungs and functional changes in respiration in animals dying after exposure. Levels and duration of exposure were not given.

Brown et al. (1969) reported the single-dose oral LD_{50} for 1,2,4-trichlorobenzene in CFE rats to be 756 mg/kg (95% confidence limits 556-939 mg/kg). In CF mice, the single-dose oral LD_{50} was 766 mg/kg (95% confidence limits 601-979 mg/kg). Death occurred within 5 days in rats and 3 days in mice.

Rimington and Ziegler (1963) studied the porphyria-inducing ability of 1,2,4- and 1,2,3-trichlorobenzenes administered by gavage to male albino rats for various time periods (5-15 days). Doses of the isomers were gradually increased until porphyrin excretion was high but fatalities were few. Porphyria was induced by 1,2,4-trichlorobenzene when the isomer was given for 15 days at 730 mg/kg (3 rats) as evidenced by peak rises in urinary coproporphyrin, uroporphyrin, porphobilinogen and δ-aminolevulinic acid. At a dose of 500 mg/kg for 10 days (in 5 rats), peak liver levels of coproporphyrin, protoporphyrin, uroporphyrin and catalase were reached. For the 1,2,3- isomer, urinary excretion of these indicators peaked at 785 mg/kg for 7 days (3 rats), but to a lesser extent than for the 1,2,4- isomer. Only the liver uroporphyrin levels were increased by administration of 1,2,3-trichlorobenzene at this dose and duration. Glutathione was found to have a protective effect on trichlorobenzene-induced porphyria.

Brown et al. (1969) determined the single-dose percutaneous LD_{50} in CFE rats (4 of each sex) to be 6139 mg/kg (95% confidence limits 4299-9056 mg/kg) for 1,2,4-trichlorobenzene administered topically and covered with an impermeable dressing. All deaths occurred within 5 days. In skin irritation studies, 1,2,4-trichlorobenzene was applied to the skin of rabbits and guinea pigs. In the first experiment, two 2x2 cm patches of lint, each containing 1 mℓ of the compound, were applied to the shorn backs of rabbits (4 of each sex) for 6 hours/day for 3 consecutive days and covered with an impermeable dressing. For another experiment, rabbits (1 of each sex) and guinea pigs (5 of each sex) received single uncovered applications of 1,2,4-trichlorobenzene (1 mℓ for rabbits, 0.5 mℓ for guinea pigs) 5 days/week for 3 weeks. The results indicated that trichlorobenzene was not very irritating, although fissuring was noted during the 3-week exposure. Some guinea pigs that died during the 3-week regimen had focal necrosis of the liver.

Hepatotoxic effects (fatty infiltration and necrosis) were reported by Cameron et al. (1937) following s.c. and/or i.v. injection of 500 mg (range of doses was 1-500 mg) trichlorobenzene in liquid paraffin to rats; the toxicity was less than that of mono- and o-dichlorobenzene. Further details of strain, number of animals or isomers were not reported.

Robinson et al. (1981), in an acute toxicity study to assess the increased adrenal weight which was noted in a multigeneration study, gave groups composed of 9-10 preweaning female Charles River rats i.p. injections of 0, 250 or 500 mg of 1,2,4-trichlorobenzene/kg in corn oil at 22, 23 and 24 days of age. Significant changes ($p<0.05$) from control values were observed upon necropsy at 25 days of age as follows: decreased body weight and increased adrenal weight at the high dose; decreased uterus and increased liver weights at both doses.

Male Holtzman rats (number not specified) were given single intraperitoneal injections of 1,2,4- or 1,3,5-trichlorobenzene at a dose of 37 mg/kg (5 mmol/kg) as a 50% solution in sesame oil in a volume of 1 mℓ/kg (Yang et al., 1979). Controls received an equal volume of sesame oil. After 24 hours, the femoral veins and the common bile duct were cannulated. Both isomers produced significant increases ($p<0.05$) in bile duct-pancreatic fluid (BDPF) flow with the 1,2,4- isomer being 4 times more effective than the 1,3,5- isomer. SGPT activity was elevated by treatment with 1,3,5-trichlorobenzene and bile flow was elevated by the 1,2,4- isomer. Both isomers caused a decrease in BDPF protein concentration.

Several studies have demonstrated the ability of the trichlorobenzenes to enhance xenobiotic metabolism. Carlson, in a series of reports (Carlson and Tardiff, 1976; Carlson, 1977a, 1978, 1981; Smith and Carlson, 1980), examined the ability of 1,2,4-trichlorobenzene to induce a variety of microsomal functions and enzymes including cytochrome c reductase, O-ethyl O-p-nitrophenyl phenylphosphothionate (EPN) detoxification, cytochrome P-450, glucuronyltransferase, benzpyrene hydroxylase and azoreductase. In a 14-day study by Carlson and Tardiff (1976), daily doses of 1,2,4-trichlorobenzene in corn oil were administered orally to groups of 6 male albino rats at 10, 20 and 40 mg/kg. All the above functions and enzymes increased significantly ($p<0.05$) except benzopyrene hydroxylase. In a 90-day study by the same investigators, all the functions and enzyme activities including benzopyrene hydroxylase increased significantly ($p<0.05$) at 10-40 mg/kg/day and remained significantly elevated after a 30-day recovery period. In a similar study, Smith and Carlson (1980) administered 1,2,4-trichlorobenzene at 181.5 mg/kg/day (1 mmol/kg/day) to rats for 7 days, and measured recovery at 1, 6, 11 and 16 days. EPN detoxification was still significantly ($p<0.05$) elevated at 11 days; p-nitroanisole demethylation at 16 days; cytochrome c reductase at 6 days; and cytochrome P-450 at 11 days. In a similar study by Carlson (1977b), 7-day administration of 1,3,5-trichlorobenzene at 100-200 mg/kg/day significantly ($p<0.05$) increased EPN detoxification, UDP glucuronyltransferase, and cytochrome c reductase, and significantly decreased hepatic G-6-P; benzpyrene hydroxylase, azoreductase and serum isocitrate dehydrogenase were not significantly affected at 200 mg/kg/day. In the same study, in vivo hepatotoxicity of carbon tetrachloride (one dose of 0.5 mℓ/kg) was significantly ($p<0.05$) enhanced by 14-day pretreatment of rats with 1,2,4-trichlorobenzene. Glucose-6-phosphatase activity was significantly ($p<0.05$) decreased by pretreatment with 1,2,4-trichlorobenzene at 5 mg/kg/day, and isocitrate dehydrogenase was decreased by pretreatment at 20 mg/kg/day.

The 1,2,4- isomer, and to a lesser extent the 1,3,5- isomer, were also shown to induce hepatic esterases (Carlson et al., 1979; Carlson, 1980). In studies similar to those previously described, rats receiving daily oral doses of 18.2 mg isomer/kg (0.1 mmol/kg) for 14 days were killed 24 hours later and hepatic microsomes were prepared. The 1,2,4-isomer was an effective inducer of both acetanilide esterase and acetanilide hydroxylase, while the 1,3,5-isomer induced only the esterase and to a lesser degree than did 1,2,4-trichlorobenzene (Carlson et al., 1979). The 1,2,4-isomer also induced hepatic arylesterase, while 1,3,5-trichlorobenzene did not (Carlson, 1980). Pretreatment of rats with 181.5 mg/kg/day (1 mmol/kg/day) of either isomer resulted in induction of procaine esterase (Carlson et al., 1979).

In a series of experiments, Ariyoshi et al. (1975a,b,c) studied the effects of the trichlorobenzenes on induction of hepatic microsomal proteins, phospholipids and enzymes, especially in relation to the activity of δ-aminolevulinic acid synthetase, the rate limiting enzyme in the biosynthesis of heme. The three trichlorobenzene isomers were administered orally to groups of 2-6 female Wistar rats at a dose of 250 mg/kg/day for 3 days, after which the rats were killed and microsomes were prepared. The results indicated that trichlorobenzenes increased the levels of microsomal proteins, phospholipids and cytochrome P-450, and enhanced the activities of aniline hydroxylase, aminopyrine demethylase and δ-aminolevulinic acid synthetase, with the 1,2,4-isomer being the most effective (Ariyoshi et al., 1975a,b). The dose response of these effects to 1,2,4-trichlorobenzene were determined (Ariyoshi et al., 1975c) for groups of 2-6 female Wistar rats treated orally with single doses of 0, 125, 250, 500, 750, 1000 and 1500 mg/kg. The results indicated that 24 hours after the administration of the isomer, microsomal protein was elevated at ≥750 mg/kg and glycogen content was decreased at ≥500 mg/kg. The activities of aminopyrine demethylase and aniline hydroxylase and the content of cytochrome P-450 were increased at ≥250 mg/kg, as was δ-aminolevulinic acid synthetase activity.

9.3.2. Subchronic Toxicity. The effects of trichlorobenzene following subchronic inhalation, as well as oral and dermal exposure, have been investigated in a variety of species. Toxicity data for the trichlorobenzenes can be found in Table 9-2.

Kociba et al. (1981) exposed 20 male Sprague-Dawley rats, 4 male New Zealand rabbits and 2 male beagle dogs by inhalation to 1,2,4-trichlorobenzene (99.4% pure) at levels of 0, 223 mg/m³ (30 ppm) or 742 mg/m³ (100 ppm) for 7 hours/day, 5 days/week for a total of 30 exposures in 44 days.

TABLE 9-2

Summary of Subchronic and Chronic Toxicity Studies on Trichlorobenzenes

Species	Route	Dose	Duration	Effects	Reference
Rat	inhalation	74.2, 742 or 7423 mg/m^3 of 1,3,5-TCB	6 hr/day, 5 day/wk for up to 13 wk	No hepatotoxicity; three high-dose rats had squamous metaplasia and focal hyperplasia of respiratory epithelium, believed to be reversible	Sasmora and Palmer, 1981
Rats, rabbits, two dogs	inhalation	223 or 742 mg/m^3 of 1,2,4-TCB	7 hr/day, 5 day/wk; total of 30 exposures in 44 days	Increase in urinary excretion of porphyria in exposed rats; increase in liver weights in high-dose rats and dogs; increased kidney weights in high-dose rats	Kociba et al., 1981
Rat	inhalation	22.3 or 74.2 mg/m^3 of 1,2,4-TCB	6 hr/day, 5 day/wk, 3 mo	Increase in urinary porphyrin excretion in high-dose rats; no effects in 22.3 mg/m^3 group	Watanabe et al., 1978
Rat	inhalation	186, 371 or 742 mg/m^3 of 1,2,4-TCB	7 hr/day, 5 day/wk, 26 wk	Enlarged hepatocytes and nondose-dependent hepatocytes vacuolization, liver granulance, biliary hyperplasia and kidney hyaline degeneration at 4 and 13 wk; no histopathology evident at 26 wk	Coate et al., 1977
Rabbits, monkeys	inhalation	186, 371 or 742 mg/m^3 of 1,2,4-TCB	7 hr/day, 5 day/wk, 26 wk	No treatment related changes at 26 wk	Coate et al., 1977
Monkey	oral	1, 5, 25, 90, 125 or 173.6 mg/kg/day of 1,2,4-TCB	30 days	$\leq$25 mg/kg/day - no effects observed; $\geq$90 mg/kg/day - observed toxicity and death	Smith et al., 1978
Rat	oral	50, 100 or 200 mg/kg/day of 1,2,4-TCB	30, 60, 90 or 120 days	Increases in liver weights, liver porphyrins and urine porphyrins, dose and time related	Carlson, 1977b
Rat	oral	10, 20 or 40 mg/kg/day of 1,2,4-TCB	90 days	Increase in liver-to-body weight ratio in high-dose group; changes in enzyme activation at all doses	Carlson and Tardiff, 1976
Mouse	oral	600 ppm diet (0.078 mg/kg/day) of 1,2,4-TCB	6 mo	No effects	Goto et al., 1972

TABLE 9-2 (cont.)

Species	Route	Dose	Duration	Effects	Reference
Guinea pig	dermal	0.5 mℓ/day of 1,2,4-TCB	5 day/wk, 3 wk	Death following extensor convulsion; livers showed necrotic foci	Brown et al., 1969
Mouse	dermal	0.003 mℓ/painting of 30 and 60% solution in acetone of 1,2,4-TCB	2 times/wk, 2 yr	Painting induced excitability, panting and epidermal thickening, inflammation and keratinization; increased organ weights and mortality	Yamamoto et al., 1957
Rats	oral (drinking water)	25, 100 or 400 mg/ℓ of 1,2,4-TCB	F_0 to F_2 generations	Enlarged adrenals in F_0 and F_1 generations	Robinson et al., 1981
Rabbits	dermal	30, 150 or 450 mg/kg/day of 1,2,3-TCB	5 day/wk, 4 wk	Dose-related skin irritation; increase in urinary coproporphyrin in high-dose males and slight pallor of liver in males and females	Rao et al., 1982

1,2,3-TCB = 1,2,3-trichlorobenzene; 1,2,4-TCB = 1,2,4-trichlorobenzene; 1,3,5-TCB = 1,3,5-trichlorobenzene

There were no significant effects on body weight, hematologic indices or serum biochemistry tests. Upon necropsy, gross and comprehensive histological examination revealed no significant treatment-related effects in any of the species. At the 742 mg/m³ level, increased liver weights were detected in dogs and rats and increased kidney weights in rats. Urinary excretion of porphyrin was increased in rats exposed to 1,2,4-trichlorobenzene at 223 or 742 mg/m³, which the investigators interpreted as a compound-specific physiologic effect rather than a toxic effect. A follow-up study supported this interpretation. The same investigators exposed male and female Sprague-Dawley rats to 1,2,4-trichlorobenzene at 0, 22.3 mg/m³ (3 ppm) or 74.2 mg/m³ (10 ppm) for 6 hours/day, 5 days/week for 3 months. The results, which were reported in an abstract (Watanabe et al., 1978), indicated that urinary excretion of porphyrins was slightly increased in the 74.2 mg/m³ group during exposure, but returned to control range 2-4 months post-exposure. Since this appeared to be the most sensitive indicator in rats, and exposure to trichlorobenzene at 22.3 mg/m³ did not cause increased porphyrin excretion, 22.3 mg/m³ was considered a no-observed-adverse-effect level (NOAEL) for rats by the authors.

Sasmore and Palmer (1981) exposed male and female outbred albino CD rats (20/group) to 1,3,5-trichlorobenzene vapor at 0, 74.2 mg/m³ (10 ppm), 742 mg/m³ (100 ppm) or 7423 mg/m³ (1000 ppm) for 6 hours/day, 5 days/week for up to 13 weeks. No significant effects were observed on body weights, food consumption, standard hematologic and clinical chemistry parameters or on methemoglobin and porphyrin levels. In a subgroup of animals killed after 4 weeks of exposure, there was a significant increase in the liver-to-body weight and liver-to-brain weight ratios in the male rats of the high exposure level group, but these effects were not observed at 13 weeks.

Since gross and microscopic pathologic examinations of the liver revealed no treatment-related abnormalities, the authors concluded that the exposure did not cause hepatotoxicity. Microscopic examinations, however, revealed that three high dose rats had squamous metaplasia and focal hyperplasia of the respiratory epithelium, which the authors believed to be reversible.

Coate et al. (1977) exposed groups of 30 male Sprague-Dawley rats, 16 male New Zealand rabbits and 9 male monkeys (Macaca fascicularis) to 99.07% pure 1,2,4-trichlorobenzene vapor at levels of 0, 186 mg/m^3 (25 ppm), 371 mg/m^3 (50 ppm) or 742 mg/m^3 (100 ppm) for 7 hours/day, 5 days/week for 26 weeks. Pulmonary function and operant behavior tests in the monkeys, ophthalmic examinations in the rabbits and monkeys, and measurements of body weight, hematologic indices and serum biochemistry parameters in all species were conducted before and during the exposure period. Subgroups of 5 rats each were killed after 4 and 13 weeks of exposure; all remaining rats were killed after 26 weeks for histological examination of selected tissues. No treatment-related effects at any observation time were seen with respect to body weight, survival, hematology or serum chemistry for any of the species. No ophthalmic changes were observed in rabbits or monkeys. Pulmonary function and operant behavior were unaffected in monkeys. Histological examination of rat tissues revealed that treated animals had enlarged hepatocytes that were more prominent at 4 weeks than at 13 weeks after exposure, and at 371 and 742 mg/m^3 than at 186 mg/m^3. Other changes in treated rats that did not appear to be dose-dependent were vacuolization of hepatocytes at 4 and 13 weeks, slightly more severe granuloma of the liver at 4 weeks and biliary hyperplasia at 4 and 13 weeks. A nondose-related increase in the severity of kidney hyaline degeneration was observed in test rats at 4 weeks. This lesion was slightly more severe in the high dose group at 13 weeks. These effects appeared to be transient; rats necropsied after 26 weeks of exposure had none of these changes. Likewise, histological examination of selected tissues from rabbits and monkeys revealed no treatment-related changes after 26 weeks of exposure.

Carlson and Tardiff (1976) assessed the effects of 14- or 90-day oral administration of 1,2,4-trichlorobenzene in corn oil compared to corn oil controls in male CD rats. In the 14-day studies, the effects examined were lethality, hepatotoxicity and the influence on hexabarbital sleeping time and other parameters of xenobiotic metabolism. A dose of 600 mg/kg, the highest dose administered, caused no deaths during the 14-day administration period. Hepatotoxicity was evaluated by dosing at 0, 150, 300 or 600 mg/kg/day and determining serum isocitrate dehydrogenase and liver glucose-6-phosphatase activities. Although no dose-related changes in serum isocitrate dehydrogenase activity was observed, liver glucose-6-phosphatase activity was significantly decreased at $\geq$300 mg/kg ($p<0.05$). Hexabarbital sleeping time was significantly decreased at 600 mg/kg/day (the only dose examined); this effect persisted through a 14-day recovery period. In rats receiving 14 daily doses at 0, 10, 20 or 40 mg/kg, there was a significant dose-related increase in liver-to-body weight ratio at $\geq$10 mg/kg/day ($p<0.05$). Significant dose-related increases were also observed in activities or contents of cytochrome c reductase (at $\geq$10 mg/kg), cytochrome P-450 (at $\geq$20 mg/kg), glucuronyltransferase (at $\geq$20 mg/kg), azoreductase (at $\geq$10 mg/kg) and the rate of detoxication of EPN (at $\geq$10 mg/kg). These results indicated that the doses, while causing a slight degree of hepatic injury, significantly enhanced xenobiotic metabolism.

In 90-day studies by Carlson and Tardiff (1976), the effects of oral dosing of male CD rats (6 animals/group) at 0, 10, 20 or 40 mg/kg/day with

1,2,4-trichlorobenzene in corn oil on weight gain, liver weight, hemoglobin content, packed cell volume and the indicators of xenobiotic metabolism were evaluated. No effects on weight gain and no consistent alteration in hemoglobin content or packed cell volume were observed. At 40 mg/kg, there was a statistically significant increase ($p<0.05$) in liver-to-body weight ratios that persisted throughout a 30-day recovery period. Following 90-day administration, cytochrome c reductase activity was increased at $\geq$10 mg/kg, with recovery after 30 days; cytochrome P-450 levels increased at $\geq$20 mg/kg, followed by recovery; glucuronyltransferase activity decreased at $\geq$10 mg/kg; EPN detoxication increased at $\geq$20 mg/kg; benzopyrene hydroxylase activity increased 2-fold at 40 mg/kg; and azoreductase activity increased at $\geq$10 mg/kg.

Groups of 5 female rats (strain not reported) received daily oral doses of 0, 50, 100 or 200 mg 1,2,4-trichlorobenzene/kg/day in corn oil for 30, 60, 90 or 120 days (Carlson, 1977b). Significant increases were observed in liver porphyrins at $\geq$100 mg/kg after 30 days exposure and in urinary porphyrins at 200 mg/kg after 30 days. For the 30-day study, slight but significant increases were also observed in liver weights at 200 mg/kg. When the compound was administered for 60 days, only the liver weights were increased. The administration of 1,2,4-trichlorobenzene for 90 days resulted in slight but significant increases in liver weights at $\geq$50 mg/kg, in liver porphyrins at $\geq$100 mg/kg and in urine porphyrins at 200 mg/kg. A significant increase was observed for liver porphyrins when the compound was given at $\geq$50 mg/kg for 120 days. The excretion of δ-aminolevulinic acid and porphobilinogen in the urine was not increased at any dose given for any duration. When the author compared the 1,2,4-trichlorobenzene results with the results for hexachlorobenzene, he concluded that trichlorobenzene induced porphyria was very small compared to the hexachlorobenzene induced porphyria (Carlson, 1977b).

A 90-day oral study by Smith et al. (1978), reported in an abstract, was reviewed by U.S. EPA (1980b), who gave further details of the study after communication with the authors. Rhesus monkeys (4/group) were given 1,2,4-trichlorobenzene in daily oral doses of 1, 5, 25, 90, 125 or 173.6 mg/kg. No toxic effects were observed at <25 mg/kg, while doses of ≥90 mg/kg were observed to be toxic, and the 173.6 mg/kg dose was lethal within 20-30 days. There were no deaths observed in the 1, 5 and 25 mg/kg groups; one death occurred in each of the 90 mg/kg and 125 mg/kg groups and two deaths occured in the 173.6 mg/kg group. Animals on the highest dose exhibited severe weight loss and predeath find tremors. All of the animals in the highest dose group had elevated BUN, Na^+, K^+, CPK, SGOT, SGPT, LDH and alkaline phosphatase as well as hypercalcemia and hyperphosphatemia from 30 days on. Smith et al. (1978) have been using the urinary pattern of chlorguanide metabolites as an indication of cytochrome P-450 dependent drug metabolism. At the high doses, monkeys showed evidence of the hepatic induction as well as increased clearance of i.v. doses of labeled TCB. Further information on the study (Smith, 1979) gave evidence of liver enzyme induction in the 90, 125 and 174 mg/kg animals. There were some pathological changes noted in the livers of the high dose groups, primarily a fatty infiltration. The point at which there was no effect related to the compound was at the 5 mg/kg level. Since only an abstract of this study was available and since the interpretation of this study was complicated by the use of other drugs and weight losses in the control animals, a valid no-observed-effect level (NOEL) cannot be derived from these data.

Two studies have assessed the dermal toxicity of the trichlorobenzenes. Powers et al. (1975) applied technical grade 1,2,4-trichlorobenzene at concentrations of 5 or 25% in petroleum ether, or 100% 1,2,4-trichlorobenzene topically in 0.2 mℓ volumes to the ventral surface of the ears of New Zealand rabbits (groups of 12 each), 3 times weekly for 13 weeks; a control group received petroleum ether only. Rabbits exposed to 5% trichlorobenzene and controls had slight redness and scaling. Dermal responses at 25 and 100% of the compound included slight to severe erythema, severe scaling, desquamation, encrustation, and some hair loss and scarring. The responses were characterized by acanthosis and keratosis, typical of moderate to severe irritation and probably attributable to degreasing action. No overt signs of systemic toxicity were noted, body weight gain was comparable in all groups, and none of the animals showed meaningful changes in gross pathology. The investigators noted that this contrasted with the findings of Brown et al. (1969), who reported that some guinea pigs, exposed topically to 1,2,4-trichlorobenzene at 0.5 mℓ/day, 5 days/week for 3 weeks, died following extensor convulsions and their livers showed necrotic foci. This difference in results may be attributed to the site of application (Brown et al., 1969, used the dorsal midline for application, a more extensive exposure site), the volume applied (0.5 mℓ vs. 0.2 mℓ), the species used, and more frequent (5 times/week vs. 3 times/week) application, although the total number of exposures was less (5x3 weeks vs. 3x13 weeks).

Rao et al. (1982) applied technical grade trichlorobenzene [1,2,4- (70%) and 1,2,3-trichlorobenzene (30%)] 5 days/week for 4 weeks, at doses of 0, 30, 150 or 450 mg/kg/day, to the dorsal skin (4x4 inch area) of groups (5 of each sex) of New Zealand rabbits weighing ~3 kg. One rabbit died after 18 applications, but the investigators were unable to determine the cause of death by either gross or histologic examination. Gross and histologic examination of the skin showed evidence of moderate irritation at the highest dose and less irritation at the lower doses. This irritation evidence consisted of epidermal scaling, thickening, fissures, ulcers and erythema. No treatment-related change was observed in clinical chemistry (BUN, glucose, SGPT, SAP) or hematology. A slight but significant increase in urinary coproporphyrin was observed in high-dose males (450 mg/kg/day) at day 24; none was seen in females. This slight porphyria and a slight generalized pallor of the liver (3/5 males, 4/4 females) were the only signs of systemic toxicity. Extensive histologic examination of numerous tissues failed to show any treatment-related abnormalities. The volume of trichlorobenzene applied at the dose levels in this study can be calculated as ≃0.06 mℓ (30 mg/kg), 0.31 mℓ (150 mg/kg) and 0.93 mℓ(450 mg/kg) by multiplying the dose in g/kg by the weight of the rabbits (3 kg) and dividing by the density of trichlorobenzene (1.45).

9.3.3. Chronic Toxicity. No studies on the effects of the trichlorobenzenes following chronic inhalation exposure were available for review; however, a chronic skin painting study was encountered. Goto et al. (1972) conducted a 6-month feeding study in mice using hexachlorocyclohexane isomers and their metabolites, including 1,2,4-trichlorobenzene. Male mice (20/group) of the ICR-JCL strain (age at initiation 5 weeks, average weight 26.5 g) received a diet containing 600 ppm of trichlorobenzene (78 μg of compound/kg body weight, assuming mice consume 13% of their body weight in food per day). The weight gain of treated mice did not differ from controls during the 6-month exposure. At 26 weeks, 10 mice were killed and liver, heart and kidneys were weighed; no abnormal weight changes were observed. Macroscopic and histologic examination of the liver revealed no hepatic tumors or any other lesions.

Yamamoto et al. (1957) studied the toxicity of 1,2,4-trichlorobenzene when painted on the skin of Slc:ddy mice 2 times/week for 2 years. Groups consisted of 75 mice/sex receiving 0.03 mℓ applications of the compound as 30 or 60% solutions in acetone. Controls consisted of 50 mice/sex and received only acetone. The skin painting produced general symptoms of excitability and panting, local skin thickening, keratinization and inflammation of the epidermis. These effects were not observed in controls. For the 30% trichlorobenzene groups, mortality was increased in females (5/75 survived for 83 weeks compared with 11/50 controls). The mean survival days were 357±125.4 for treated females compared with 423.8±145.0 for controls ($p<0.01$). The survival of males at this exposure level was not significantly different from that of controls. Spleen weights were significantly increased ($p<0.05$) and left adrenal weights were significantly decreased ($p<0.01$) for treated males when compared with controls. Hematologic and blood chemistry indices were essentially unchanged with the exception of increased red blood cell counts in treated males ($p<0.05$) and decreased Cl^- concentration ($p<0.01$). For the 60% solution, 6/75 treated females survived for 83 weeks. Mean survival days were 320.2±147.7 for treated females compared with 423.8±145.0 for controls ($p<0.001$). Eight of 75 treated males survived for 83 weeks compared with 9/50 control males. Mean survival days were 288.0±173.7 for treated males and 363.9±173.9 for controls ($p<0.05$). Significant differences in organ weights from control values were seen in the spleens of males ($p<0.01$) and the adrenals of females ($p<0.05$). Hematologic and blood biochemistry changes were seen in increased lymphocyte counts in treated females ($p<0.05$), and in increased SGOT ($p<0.05$), SGPT ($p<0.001$) and BUN ($p<0.01$) for treated males.

9.3.4. Mutagenicity. Schoeny et al. (1979) and Lawlor et al. (1979) examined the mutagenic potential of 1,2,4-trichlorobenzene in Salmonella typhimurium tester strains TA98, TA100, TA1535 and TA1537, using the plate incorporation technique. Schoeny et al. (1979) used 8 concentrations of trichlorobenzene ranging from 102 μg/plate to 1.4x10^5 μg/plate. The toxic dose was determined as 1599 μg/plate (killing of one or more strain on mutagenesis plates). Trichlorobenzene was negative for mutagenicity in the absence and presence of S-9 microsomal fractions from uninduced rats, from rats induced by the polychlorinated biphenyl, Aroclor 1254, and from rats homologously induced with trichlorobenzene.

The study of Lawlor et al. (1979), reported in an abstract, used the TA1538 strain of S. typhimurium in addition to the strains previously mentioned. Negative results were obtained for five unspecified concentrations tested in the presence and absence of rat liver microsomes induced by Aroclor 1254. Because these results were reported in an abstract without the details of the experimental procedures used, the results cannot be critically evaluated.

The negative results in the Salmonella histidine reversion assay are not unexpected because this test system is generally insensitive to chlorinated compounds.

9.3.5. Carcinogenicity. Yamamoto et al. (1957) applied 1,2,4-trichlorobenzene in acetone to the skin of Slc.ddy mice 2 times/week for 2 years. The solution of 1,2,4-trichlorobenzene was 60% for the high dose and 30% for the low dose and the volume applied was 0.03 mℓ/application. Each treated group contained 75 animals and there were 50 control animals for each sex. Growth rates in treated and control mice were comparable through 83 weeks. Mean survival days were significantly reduced in the 60% 1,2,4-trichlorobenzene groups of males and females and also in the 30% treatment group of females.

Histopathology showed some organs sites had increased non-neoplastic lesions. Assuming that all 75 animals in the treated groups were examined and all 50 in the control groups were examined, there would be increases in lesions in the males in lung, liver, kidney, adrenal, spleen and lymph node at the high dose, and in all of these organs except lymph node in the females at the high dose. Unfortunately, the English translation of Japanese text is not very specific in describing the nature of the lesion making it difficult to use this information in the interpretation of the tumor findings.

No single tumor type was increased significantly over the control incidence but among males nine different tumors were found in the high dose group as compared with two in the low dose and two in the control group. In females there were 11 different tumors in the high dose group as compared with three in the low dose and eight in the control group. The authors do not state whether these tumors were all found in different individual animals or whether these were multiple tumors in the same animal. Therefore, the actual incidence in terms of the number of tumor bearing animals is not known.

Further information from this study is necessary for full interpretation. This single study is clearly inadequate for making any conclusions about carcinogenicity in humans.

9.3.6. Reproductive and Teratogenic Toxicity. Studies on the reproductive or teratogenic effects of trichlorobenzenes following inhalation exposure were not found in the available literature. Robinson et al. (1981) reported a multigeneration study of the reproductive effects of 1,2,4-trichlorobenzene following oral administration. Charles River rats were continuously exposed to the compound at 0, 25, 100 or 400 ppm in drinking water. The authors calculated the dosages for the F_0 generation based on water consumption data to be: for females at 29 days of age, 8.3±0.8, 28.0±1.2, 133.2±13.4 mg/kg/day, respectively; for males at 29 days of age, 8.5±0.6, 27.6±1.6, 133.6±15.6 mg/kg/day, respectively; for females at 83 days of age, 3.7±0.1, 14.8±1.0, 53.6±3.9 mg/kg/day, respectively; for males at 83 days of age, 2.5±0.1, 8.9±0.3, 33.0±1.4 mg/kg/day, respectively. The exposure period began with the birth of the F_0 generation and continued through 32 days of age of the F_2 generation. Each treatment group consisted of 17-23 litters. No treatment-related effects were noted with respect to fertility, neonatal weights, maternal weights, litter sizes, preweaning viability or postweaning growth in any generation. Treatment-related differences were seen with respect to food intake and water consumption in F_0 males and females, but they were inconsistent and did not occur in other generations. Blood chemistry analyses and locomotor activity measurements revealed no overt hematologic or neurologic effects, and histological examination of the livers and kidneys of the F_1 generation rats revealed no damage. At the 400 ppm dose level, significantly enlarged adrenals in both sexes of the F_0 and F_1 rats were observed at 95 days of age ($p<0.006$). A follow-up acute toxicity study showed that this effect could result from three daily i.p. injections of 500 mg 1,2,4-trichlorobenzene/kg.

Black et al. (1983) reported in an abstract a teratogenicity study in pregnant Wistar rats using 1,2,4-, 1,2,3- or 1,3,5-trichlorobenzene administered by gavage in doses of 75-600 mg/kg on days 6-15 of gestation (gestational day 0 or 1 not defined). Upon necropsy (gestational day not specified), thyroid and liver lesions and reduced hemoglobin and hematocrit values were observed in treated dams (doses not specified). No teratogenic effects were observed in the pups; however, pups exposed to the 1,2,4- and 1,3,5- isomers (doses not specified) had mild osteogenic changes.

9.4. INTERACTIONS

Several studies discussed in Section 9.3.1. on acute toxicity have demonstrated that the isomers of trichlorobenzene are capable of affecting xenobiotic metabolism by inducing a variety of the hepatic drug-metabolizing enzymes in rats. These include cytochrome c reductase, cytochrome P-450, glucuronyltransferase, benzpyrene hydroxylase, azoreductase (Carlson and Tardiff, 1976; Carlson, 1977, 1978, 1981; Smith and Carlson, 1980), acetanilide esterase and acetanilide hydroxylase, procaine esterase (Carlson et al., 1979), arylesterase (Carlson, 1980), microsomal proteins, phospholipids and aminopyrene hydroxylase (Ariyoshi et al., 1975a,b,c). That trichlorobenzenes enhance xenobiotic metabolism has been demonstrated by Smith and Carlson (1980) and Carlson (1977a), who showed that administration of 1,2,4- or 1,3,5-trichlorobenzene to groups of 4 male Sprague-Dawley rats for 7 days increased EPN detoxication.

Townsend and Carlson (1981) demonstrated that 1,2,4-trichlorobenzene, administered by gavage in corn oil to groups of five male Swiss mice at 181.5 mg/kg (1 mmol/kg) for 7 days, increased the LD_{50} and protected the mice against the toxic effects of malathion, malaoxon, parathion and paraoxon when graded doses of these insecticides were administered on the day following the last dose of trichlorobenzene.

Experiments comparing the effects of trichlorobenzenes with the effects of phenobarbital and 3-methylcholanthrene indicated that the inductions of microsomal enzymes by trichlorobenzenes are of the phenobarbital type (Carlson, 1978).

9.5. SUMMARY

The trichlorobenzenes appear to enter the body readily via inhalation, ingestion and dermal absorption; however, data were not available to quantitate the rates of these processes nor of any of the pharmacokinetic processes. Initial distribution of the trichlorobenzenes and metabolites is mainly to the liver, kidneys and adrenals, followed by migration to adipose tissue or metabolism to polar compounds that are more readily excreted. Metabolism appears to be initially to arene oxides, and then by different routes in different species, with different rates of excretion. Species differences are such that extrapolation of adverse effects to humans probably requires the support of comparative metabolic data.

Human exposure to 1,2,4-trichlorobenzene at 3-5 ppm causes eye and respiratory irritation (Rowe, 1975). The only other data on human exposure are individual case reports of aplastic anemia of persons exposed occupationally or domestically (Girard et al., 1969).

The effects in mammals of acute exposure by various routes to trichlorobenzenes include local irritation, convulsions and death. Livers, kidneys, adrenals, mucous membranes and brain ganglion cells appear to be target organs with effects including edema, necrosis, fatty infiltration of livers, increased organ weights, porphyrin induction and microsomal enzyme induction.

Quantitative data on the toxic effects of trichlorobenzene following subchronic exposure by various routes were obtained in a variety of species. In general, these studies indicate that the liver and kidney are target organs. Inhalation of 1,2,4-trichlorobenzene at ≥ 74.2 mg/m³ (10 ppm) for 6 hours/day, 5 days/week for up to 26 weeks induced hepatocytomegaly and hyaline degeneration in several species (Kociba et al., 1981; Watanabe et al., 1978; Coate et al., 1977), although these effects may be to some extent reversible. One study (Watanabe et al., 1978) identified 22.3 mg/m³ (3 ppm) as a NOAEL in rats. Sasmore and Palmer (1981) reported that some rats exposed by inhalation to 1,3,5-trichlorobenzene at 7423 mg/m³

(1000 ppm) for 13 weeks showed squamous metaplasia and focal hyperplasia of the respiratory epithelium, which appeared to be reversible. Subchronic oral studies have also found that the trichlorobenzenes induce hepatic xenobiotic metabolism (Carlson and Tardiff, 1976; Smith et al., 1978) and porphyria (Carlson, 1977b). Subchronic dermal exposure resulted in mild to moderate irritation (Powers et al., 1975; Rao et al., 1982).

One chronic study, on the effects of trichlorobenzene painted on the skin of mice for 2 years, reported increased mortality in females at the low dose (30% solution in acetone) and in both sexes at the high dose (60% solution) (Yamamoto et al., 1957). No tumorigenic effects were seen.

Results of two reports on mutagenicity tests with Salmonella typhimurium test strains were negative (Schoeny et al., 1979; Lawlor et al., 1979). However, this test system is generally insensitive to chlorinated compounds. A multigeneration study of the reproductive effects of oral exposure to trichlorobenzene (Robinson et al., 1981) and an oral teratogenicity study in rats (Black et al., 1983) failed to show effects on reproduction or fetal development, although pups had mild osteogenic changes.

10. TETRACHLOROBENZENES

Approximately 50 million pounds of the three tetrachlorobenzene isomers were produced annually in the United States, and the 1,2,4,5- isomer was produced the most (U.S. EPA, 1981). Recent information indicates that the production of the tetrachlorobenzenes have been substantially reduced or have been stopped for the present time (U.S. EPA, 1983). The 1,2,3,4- and 1,2,3,5- isomers have limited use industrially and are byproducts in the synthesis of 1,2,4,5-tetrachlorobenzene. The 1,2,4,5- isomer [illegible] primarily used as an intermediate in the synthesis of fungicides, bactericides and herbicides (see Sections 4.1. and 4.2.) (U.S. EPA, 1977). Tetrachlorobenzene isomers have been detected in environmental samples as well as in human tissues and breath, but no quantitative exposure assessment has been completed (see Sections 4.3. and 4.4.). Fish and other organisms bioaccumulate the tetrachlorobenzenes, indicating that human exposure from the food chain is likely along with human atmospheric exposure (see Section 4.4.).

10.1. PHARMACOKINETICS

No studies describing the absorption, distribution, metabolism or excretion of 1,2,3,4-, 1,2,3,5- or 1,2,4,5-tetrachlorobenzene following inhalation exposure were located in the available literature. Several oral studies describing the pharmacokinetics of the three tetrachlorobenzene isomers in rats, rabbits and dogs are available and are discussed in detail below.

10.1.1. Absorption. Jondorf et al. (1958) examined the absorption of 1,2,3,4-, 1,2,3,5- and 1,2,4,5-tetrachlorobenzene from the gastrointestinal tract of female Chinchilla rabbits. Groups of three rabbits were given a single dose of the tetrachlorobenzene isomers by stomach tube at a dose level of 500 mg/kg in a 10% solution in arachis oil. Through 6 days postdosing, the percentages of the administered doses recovered in the feces as the intact compound were 5% for 1,2,3,4-tetrachlorobenzene, 14% for 1,2,3,5-tetrachlorobenzene and 16% for 1,2,4,5-tetrachlorobenzene. Considering the small amount of isomers in the feces through 6 days postdosing and that some of this fecal content may have been due to biliary excretion, it can be assumed that gastrointestinal absorption of the three tetrachlorobenzene isomers is a relatively efficient process in rabbits (Jondorf et al., 1958). The percentages of the administered doses recovered unchanged in the "gut contents" were 0.5, 1.4 and 6.2% for 1,2,3,4-, 1,2,3,5- and 1,2,4,5-tetrachlorobenzene, respectively, suggesting that the chlorine positions on the molecule may influence absorption. Based on the microanatomy of the respiratory epithelia, a similar absorption efficiency may be predicted for inhalation exposures but the efficiency of gastrointestinal absorption may also be due to enterohepatic recycling.

10.1.2. **Distribution.** The tissue distribution patterns of 1,2,4,5-tetrachlorobenzene in beagle dogs (Braun et al., 1978) and of all three tetrachlorobenzene isomers in Chinchilla rabbits (Jondorf et al., 1958) and rats (Chu et al., 1983; Jacobs et al., 1977) have been described. None of these investigators speculated on comparisons between the animal species tested and humans.

Braun et al. (1978) administered 5 mg/kg/day of 1,2,4,5-tetrachlorobenzene in the diet to 2 male and 2 female beagle dogs for 2 years. The resulting distribution of 1,2,4,5-tetrachlorobenzene was described in terms of a two-compartment pharmacokinetic model, with clearance rate constants (k_e) of $6.64 \pm 0.82 \times 10^{-3}$ day^{-1} for plasma and $6.22 \pm 0.58 \times 10^{-3}$ day^{-1} for fat tissue. The half-lives for elimination from fat and plasma were 111 and 104 days, respectively. The authors concluded that steady-state was approached at a faster rate in fat than in plasma. However, the steady-state profiles for both fat and plasma (Tables 10-1 and 10-2) appear to be similar, and no statistically significant difference was reported. The fat:plasma ratio (F/P) was ~650 after 1 month of treatment, indicating that 1,2,4,5-tetrachlorobenzene has a high affinity for fat. During the remainder of the study, F/P decreased steadily, reaching ~280 by the end of the study. Therefore, the fat was probably becoming saturated with each successive dose, and the 1,2,4,5-tetrachlorobenzene concentration in the plasma increased more rapidly over time than that in the fat. During the 20-month observation period that followed treatment, F/P increased rapidly, as the available 1,2,4,5-tetrachlorobenzene in the plasma and other hypothetical low affinity compartments was preferentially redistributed to the high affinity fat compartment.

Jondorf et al. (1958) administered dosages of 500 mg/kg each of the three tetrachlorobenzene isomers in a 10% solution in arachis oil by stomach tube to groups of three Chinchilla rabbits. The animals were killed 6 days post-dosing, and the unchanged tetrachlorobenzene isomers were detected in the liver, brain, skin, depot fat, gut contents and other unspecified parts of the body (cumulatively referred to as "rest of body"). The percentage of the administered dose measured as unchanged isomer for each of the above tissues is presented in Table 10-3.

Chu et al. (1983) administered ^{14}C-labeled 1,2,3,4-, 1,2,3,5- and 1,2,4,5-tetrachlorobenzene as a single oral dose of 1 or 10 mg/kg to male rats, and killed the treated animals 7 days postdosing. At the higher dose level, 1,2,4,5-tetrachlorobenzene was observed in all tissues examined, including fat (411 ppm), skin (33 ppm), kidney (23 ppm) and liver (22 ppm); there was no indication in the abstract whether these concentrations were

TABLE 10-1

Percentage of 1,2,4,5-Tetrachlorobenzene Steady-State Reached at Specific Times in Fat and Plasma of Dogs*

Time of Exposure (days)	Percentage of Steady-State Profile	
	Fat	Plasma
10	5.5	4.8
30	16	14
90	40	35
180	64	58
365	87	83
730	98	97

*Source: Braun et al., 1978

2 Male and 2 female beagle dogs were administered 5 mg/kg/day of 1,2,4,5-tetrachlorobenzene in the diet.

TABLE 10-2

Time Required to Reach Various Percentages of 1,2,4,5-Tetrachlorobenzene Steady-State in Fat and Plasma of Dogs*

Percentage of Steady State	Time (days)	
	Fat	Plasma
99.9	1220	1418
98.0	691	803
90.0	407	473
50.0	122	142

*Source: Braun et al., 1978

2 Male and 2 female beagle dogs were administered 5 mg/kg/day of 1,2,4,5-tetrachlorobenzene in the diet.

TABLE 10-3

Unchanged Tetrachlorobenzene in Rabbit Tissues
6 Days After Oral Dosing (500 mg/kg)*

Tetrachlorobenzene Isomer	Percentage of Dose						
	Liver	Brain	Skin	Depot Fat	Gut Contents	Rest of Body	Total
1,2,3,4-	0.1	0	2	5	0.5	2.0	10
1,2,3,5-	<0.5	<0.2	5	11	1.4	5.2	23
1,2,4,5-	0.1	<0.1	10	25	6.2	6.4	48

*Source: Jondorf et al., 1958

intact compound or radioactivity. Similar tissue distribution patterns were observed for animals given the higher doses of 1,2,3,4- or 1,2,3,5-tetrachlorobenzene, but tissue concentrations were much less; further detail regarding target tissues and concentrations for these two isomers were not reported in the abstract. At the lower doses, a similar tissue distribution pattern for all three isomers was observed.

As reported in the summary of a German study, Jacobs et al. (1977) continuously fed rats diets containing 1,2,4,5-tetrachlorobenzene (dose level and duration not reported). Accumulation of 1,2,4,5-tetrachlorobenzene and its derivatives were greatest in adipose tissue. The maximum concentrations in adipose tissue and blood were reached by 3 weeks after initiation of treatment, and steady state was attained in both adipose and blood compartments by 5 weeks after initiation of treatment.

Morita et al. (1975d) analyzed adipose tissue samples of 15 residents of the Tokyo metropolitan area for 1,2,4,5-tetrachlorobenzene. Residual tissue levels of 1,2,4,5-tetrachlorobenzene ranged from 0.006-0.039 μg/g of fat, with a mean residual tissue level of 0.019 μg/g of fat. The source and route of exposure to 1,2,4,5-tetrachlorobenzene were not identified.

10.1.3. **Metabolism.** Kohli et al. (1976a) examined the metabolic fate of the three tetrachlorobenzene isomers in male rabbits following a single intraperitoneal injection of the compounds dissolved in vegetable oil at dose levels of 60-75 mg/kg. The urine and feces of the treated animals were collected for 10 days postdosing and examined for major metabolites. 1,2,3,5-Tetrachlorobenzene was the most extensively metabolized isomer, yielding 2,3,4,5-, 2,3,5,6- and 2,3,4,6-tetrachlorophenol. 1,2,3,4-Tetrachlorobenzene was metabolized to 2,3,4,5- and 2,3,4,6-tetrachlorophenol, while 1,2,4,5-tetrachlorobenzene yielded the single metabolite, 2,3,5,6-tetrachlorophenol. The authors proposed corresponding arene oxides as electrophilic intermediate metabolites of all three tetrachlorobenzene isomers, with the ultimate tetrachlorophenol formation from 1,2,3,5- and 1,2,3,4-tetrachlorobenzene involving an NIH shift of a chlorine atom. The metabolism of 1,2,4,5-tetrachlorobenzene to 2,3,5,6-tetrachlorophenol can be achieved via the 2,3,5,6-tetrachlorobenzene oxide intermediate without an NIH shift of a chlorine atom. Further evidence of this metabolic pathway was provided by Ariyoshi et al. (1974, 1975a,b), who reported that all three tetrachlorobenzene isomers increased the cytochrome P-450 enzyme activity in the liver of rats, indicating that oxidative metabolism with the formation of the corresponding arene oxide intermediate is a plausible pathway.

The metabolic fate of the tetrachlorobenzenes in rabbits also was investigated by Jondorf et al. (1958). Single doses of 500 mg/kg tetrachlorobenzene isomers were given to groups of three rabbits by stomach tube in a 10% solution in arachis oil. The metabolic products detected in the urine through day 6 postdosing, as summarized in Table 10-4, included tetrachlorophenols and the glucuronide, ethereal sulfate and mercapturic acid conjugates. The authors suggested that the tetrachlorobenzenes were metabolized via the competitive reactions involving oxidative hydroxylation or reductive dechlorination. In agreement with the results obtained by Kohli et al (1976a), Jondorf et al. (1958) also reported that 1,2,4,5-tetrachlorobenzene was the least metabolized tetrachlorobenzene isomer; 48% of the administered dose of 1,2,4,5-tetrachlorobenzene was detected as the intact compound in the tissues of rabbits at 6 days after administration, as compared to 10% for 1,2,3,4-tetrachlorobenzene and 23% for 1,2,3,5-tetrachlorobenzene. It

TABLE 10-4

Urinary Metabolites of Tetrachlorobenzene Isomers in Rabbits 6 Days After Oral Dosing (500 mg/kg)*

Tetrachlorobenzene Isomer	Percentage of Dose (Mean Values) Excreted as				
	Glucuronide	Ethereal Sulfate	Mercapturic Acid	Tetrachlorophenol	
				Free	Total
1,2,3,4-	30	3	<1	8	43
1,2,3,5-	6	2	0	1.9	5
1,2,4,5-	4	1	0	1.3	2.2

*Source: Jondorf et al., 1958

was suggested by Morita (1977) that the metabolism of 1,2,4,5-tetrachlorobenzene via oxidative hydroxylation is partially inhibited because of steric factors.

Chu et al. (1983) administered single oral doses of 1 or 10 mg/kg ^{14}C-labeled 1,2,4,5-, 1,2,3,5- and 1,2,3,4-tetrachlorobenzene each to male rats, and killed the treated animals 7 days postdosing. Urinary metabolites of the tetrachlorobenzene isomers detected included tetrachlorophenols, trichlorophenols, dihydroxylated tetrachlorobenzenes and trace amounts of sulfur-containing metabolites; no distinction between individual isomers and metabolites was made in the abstract.

The tetrachlorobenzenes have been reported as metabolites of lindane in rats (Engst et al., 1976a), molds (Engst et al., 1979), hen pheasants, wheat, lettuce and endives (Kohli et al, 1976b,c; Saha and Burrage, 1976), and of hexachlorobenzene in rats (Mehendale et al., 1975; Engst et al., 1976a).

10.1.4. Excretion. Jondorf et al. (1958) administered single doses of 500 mg/kg each of the tetrachlorobenzene isomers to groups of three rabbits by stomach tube in a 10% solution in arachis oil. The tetrachlorobenzene isomers were excreted as phenols (primarily tetrachlorophenols) in the urine, as intact compound in the feces and breath and as other chlorobenzenes in the expired air. Total excretion of the administered dose at 6 days postdosing was 68% for both 1,2,3,4- and 1,2,3,5-tetrachlorobenzene, and 83% for 1,2,4,5-tetrachlorobenzene. The excretion profiles for the isomeric tetrachlorobenzenes are summarized in Table 10-5, and the excretion of the intact compound in the expired air over 5 days postdosing is summarized in Table 10-6.

TABLE 10-5

Summary of Excretion of the Isomeric Tetrachlorobenzenes as Metabolites or as Unchanged Compound in Rabbits Dosed Orally (500 mg/kg)*

Tetrachlorophenol Isomer	Percentage of Dose Excreted as						
	Phenols in Urine		Unchanged Tetrachlorobenzene in				
	Tetrachlorophenols	Other Phenols	Feces	Tissues	Breath	Other Chlorobenzenes in Breath	Total
1,2,3,4-	43	<1	5	10	8	2	68
1,2,3,5-	5	5	14	23	12	9	68
1,2,4,5-	2	5	16	48	2	10	83

*Source: Jondorf et al., 1958

TABLE 10-6

Excretion of Unchanged Tetrachlorobenzenes in the Expired Air of Rabbits After Oral Dosing (500 mg/kg)*

Tetrachlorobenzene Isomer	Percentage of Dose in Expired Air					
	Days after Dosing					Total
	1	2	3	4	5	
1,2,3,4-	1.9	2.2	1.6	0.2	0	5.9
1,2,3,5-	2.1	2.1	1.2	2.9	2.6	10.9
1,2,4,5-	1.2	0.2	0.2	0	0	1.6

*Source: Jondorf et al., 1958

Chu et al. (1983) administered single oral doses of 1 or 10 mg/kg each of ^{14}C-labeled 1,2,4,5-, 1,2,3,5- and 1,2,3,4-tetrachlorobenzene to male rats, and killed the treated animals 7 days postdosing. Animals receiving the higher dose of 1,2,4,5-tetrachlorobenzene were observed to excrete 16.7% of the administered dose in the urine and 4.8% in the feces. The percentage of the administered dose excreted in the urine and feces of animals dosed with 1,2,3,5- or 1,2,3,4-tetrachlorobenzene was greater than that for those dosed with 1,2,4,5-tetrachlorobenzene; however, actual percentages were not reported. Excretion of the lower doses of isomers were similar to the percentage values observed with the higher doses, but quantitative results were not presented in the abstract.

10.1.5. **Summary.** No studies describing the absorption, distribution, metabolism or excretion of 1,2,3,4-, 1,2,3,5- or 1,2,4,5-tetrachlorobenzene following inhalation exposure were located in the available literature. The pharmacokinetics of the tetrachlorobenzene isomers following oral administration is well characterized in rabbits, but not in other animal species. The lipophilic characteristics of the tetrachlorobenzene isomers would allow efficient transepithelial absorption at the gastrointestinal and respiratory surfaces. Once absorbed, the tetrachlorobenzene isomers administered orally to rabbits was rapidly accumulated in fat, metabolized primarily to tetrachlorophenols and conjugated partly as glucuronides and ethereal sulfates, or eliminated unchanged in the expired air or feces (Jondorf et al., 1958).

No pharmacokinetic data were available for humans, except a report of 1,2,4,5-tetrachlorobenzene in adipose tissue (range of 0.006-0.039 mg/kg bw; mean of 0.019 mg/kg bw) of 15 Tokyo residents (Morita et al., 1975d). Although quantitative estimates of human exposure to the tetrachlorobenzene isomers via air, food or drinking water were not available, based on the relatively limited industrial use of the tetrachlorobenzene isomers (U.S. EPA, 1980b), human exposure may not be significant. The tetrachlorobenzene isomers are both *in vivo* and *in vitro* metabolites of the pesticides, lindane and hexachlorobenzene (Mehendale et al., 1975; Engst et al., 1976a,b, 1979; Kohli et al, 1976b,c; Saha and Burrage, 1976); therefore, human exposure via air, food and drinking water may occur from the environmental degradation of these pesticides.

10.2. EFFECTS ON HUMANS

Only one epidemiologic study was available regarding the effects of the tetrachlorobenzenes on humans. Kiraly et al. (1979) examined peripheral lymphocytes for chromosomal abnormalities in blood collected from Hungarian workers engaged in the production of 1,2,4,5-tetrachlorobenzene. The "normal control" group consisted of 49 nonfactory workers (ages, 26-52 years; average age, 38.2 years) who provided blood for chromosome examination at a genetic counseling clinic. The "factory employees control" group consisted of 14 factory employees (ages, 28-47 years; average age, 35.4 years; duration of employment range of 10-30 years) not directly exposed to the 1,2,4,5-tetrachlorobenzene manufacturing process, but with possible inadvertent exposure to other unspecified airborne pollutants. The "positive control" group contained 25 factory workers (ages, 31-59 years; average age, 44.6 years) producing 1,2,4,5-tetrachlorobenzene; each had been employed at that job for ≥6 months, working 8 hours/day, and wearing "Tucan-type" face masks during work hours. Coded samples of peripheral lymphocytes were cultured for 48 hours, and ≥50 metaphase cells were examined for each sample. Factory air concentrations of 1,2,4,5-tetrachlorobenzene were not determined.

The group of workers exposed to 1,2,4,5-tetrachlorobenzene had a significantly increased ($p<0.01$) frequency of cells with <46 chromosomes when compared with both the normal and factory employee control groups. Polyploidy was observed in 2.94% (40 of 1360) of the mitoses examined from the exposed group, compared with 0.59% (15 of 2523) in the normal control, and 2.50% (21 of 838) in the factory control group; statistical significance was not indicated. Inadvertent exposure to airborne pollutants may have resulted in the relatively high percentage of polyploidy and chromosome aberrations observed in the factory control group. The frequencies for chromatid-type chromosome aberrations, labile chromosome-type aberrations and stable chromosome-type aberrations for the three groups are listed in Tables 10-7, 10-8 and 10-9, respectively. The authors concluded that 1,2,4,5-tetrachlorobenzene was mutagenic (i.e., clastogenic) to occupationally exposed humans.

10.3. MAMMALIAN TOXICOLOGY

No animal studies on acute toxicity, subchronic toxicity, chronic toxicity, mutagenicity, carcinogencity or reproductive and teratogenic effects of 1,2,3,4-, 1,2,3,5- or 1,2,4,5-tetrachlorobenzene following inhalation exposure were located in the available literature. Several oral studies describing some of the effects of the three tetrachlorobenzene isomers in animal species are available and are described below. A summary of subchronic, chronic, reproductive and teratogenic toxicity studies on tetrachlorobenzenes can be found in Table 10-10.

10.3.1. Acute Toxicity. The oral LD_{50} for 1,2,4,5-tetrachlorobenzene was reported to be 1035 mg/kg when given in sunflower oil and 2650 mg/kg when given in 1.5% starch solution in mice and 1500 mg/kg when given in

TABLE 10-7

Frequency of Chromatid-type Chromosome Aberrations in Peripheral Lymphocytes[a]

Parameter	Normal Control	Factory Control	1,2,4,5-Tetrachlorobenzene Exposed
No. of Mitoses Examined (subjects)	2523 (49)	838 (14)	1360 (25)
Gap			
Number	73	46	81
Percent	2.89	5.48	5.95
Isogap[b]			
Number	19	2	30
Percent	0.75	0.23	2.20
Total (Gap + Isogap)	92	48	111
Percent	3.64	5.71	8.15
Break			
Number	40	26	55
Percent	1.59	3.10	4.04
Isobreak[b]			
Number	17	18	32
Percent	0.67	2.14	2.35
Total (Break + Isobreak)	57	44	87
Percent	2.26	5.24	6.39
Exchange			
Number	0	0	2
Percent	0	0	0.15
Total Aberrations			
Number	149	92	198
Percent	5.90	10.97	14.70[c]

[a] Source: Kiraly et al., 1979

[b] Isogap and isobreak are aberrations involving the same location on two chromatids

[c] Statistically significant difference between exposed and each of the control groups; test and p value not specified.

TABLE 10-8

Frequency of Labile Chromosome-type Aberrations*

Parameter	Normal Control	Factory Control	1,2,4,5-Tetrachlorobenzene Exposed
No. of Mitoses Examined (subjects)	2523 (49)	838 (14)	1360 (25)
Acentric Fragment			
Number	9	8	19
Percent	0.35	0.95	1.40
Ring Chromosome			
Number	0	0	2
Percent	0	0	0.15
Dicentric Chromosome			
Number	0	0	2
Percent	0	0	0.15
Total			
Number	9	8	23
Percent	0.35	0.95	1.69

*Source: Kiraly et al., 1979

TABLE 10-9

Frequency of Stable Chromosome-type Aberrations[a]

Parameter	Normal Control	Factory Control	1,2,4,5-Tetrachlorobenzene Exposed
No. of Karyotypes Examined	460	144	237
Deletion			
Number	19	10	27
Percent	4.13	6.94	11.39
Inversion			
Number	4	1	4
Percent	0.87	0.69	1.68
Translocation			
Number	3	2	5
Percent	0.65	1.38	2.10
Total			
Number	26	13	36
Percent	5.65	9.02	15.18[b]

[a] Source: Kiraly et al., 1979

[b] Statistically significant difference between exposed and normal controls and factory controls ($p<0.1$, test not specified).

TABLE 10-10

Summary of Toxicity Studies on Tetrachlorobenzenes

Species	Route	Dose	Duration	Effects	Reference
Rat	oral	0.5-500 mg/kg of diet 1,2,4,5-TeCB	28 or 90 days	Increased liver and kidney weights and histological changes in liver and kidneys; increases in MFO activity, serum cholesterol values	Villeneuve et al., 1983
Rat	oral	0.001, 0.005, 0.05 mg/kg/day 1,2,4,5-TeCB	8 mo	No effects observed in 0.001 mg/kg/day dose group; 0.005 and 0.05 mg/kg/day doses caused disruption in conditioned reflexes, increases in liver weight coefficients and decrease in serum SH groups	Fomenko, 1965
Rabbit	oral	0.001, 0.005, 0.05 mg/kg/day 1,2,4,5-TeCB	8 mo	No effect observed in 0.001 mg/kg/day dose group; 0.05 mg/kg dose caused disorder of liver glycogen formation, altered serum SH group levels, increase in blood hemoglobin and peripheral reticulocyte levels	Fomenko, 1965
Rat	oral	75 mg/kg/day 1,2,4,5,-TeCB	2 mo	Altered biochemical parameters indicating changes in hepatic and hematopoitic homeostasis	Fomenko, 1965
Dog	oral	5 mg/kg/day 1,2,4,5-TeCB	2 yr exposure, 22 mo recovery	No controls used; elevated SAP and total bilirubin, returned to normal range 3 mo after exposures ended	Braun et al., 1978
Pregnant rats	oral	50, 100, 200 mg/kg/day 1,2,4,5-TeCB	days 6-15 of gestation	High-dose lethal to 9/10 of treated dams; organ weight changes, elevated serum cholesterol and liver metabolism enzymes, no indication of those changes were dose-related	Ruddick et al., 1981
Pregnant rats	oral	50, 100, 200 mg/kg/day 1,2,3,4-TeCB	days 6-15 of gestation	Induced maternal toxicity and increased lethality of pups at 200 mg/kg/day	Ruddick et al., 1981
Pregnant rats	oral	50, 100, 200 mg/kg/day 1,2,3,5-TeCB	days 6-15 of gestation	Increased lethality in 200 mg/kg/day group pups; one pup malformed and minor chondrogenic delay in other pups	Ruddick et al., 1981

1,2,4,5-TeCB = 1,2,4,5-tetrachlorobenzene
1,2,3,4-TeCB = 1,2,3,4-tetrachlorobenzene
1,2,3,5-TeCB = 1,2,3,5-tetrachlorobenzene

apparently sunflower oil in rats and rabbits (Fomenko, 1965). Villeneuve et al. (1983) reported an LD_{50} range of ~1200-3000 mg/kg in rats for the three tetrachlorobenzene isomers with 1,2,3,4-tetrachlorobenzene > 1,2,3,5-tetrachlorobenzene > 1,2,4,5-tetrachlorobenzene; further details regarding doses and effects were not provided in the abstract.

Rimington and Ziegler (1963) administered relatively large dietary doses of 1,2,3,4-tetrachlorobenzene at a level of 660 mg/kg/day for 10 days or 1,2,4,5-tetrachlorobenzene at a level of 905 mg/kg/day for 5 days to rats. 1,2,3,4-Tetrachlorobenzene induced weight loss, nonnecrotic liver cell degeneration and an increase in porphyrin and hemoglobin metabolism, while the only effect reported for 1,2,4,5-tetrachlorobenzene was nonnecrotic liver cell degeneration.

No studies were available regarding the dermal toxicity or sensitization reactions of the three tetrachlorobenzene isomers.

10.3.2. Subchronic Toxicity. As reported in an abstract, Villeneuve et al. (1983) administered dietary concentrations of 1,2,3,4-, 1,2,3,5- and 1,2,4,5-tetrachlorobenzene ranging from 0.5-500 ppm to both sexes of rats for 28 or 90 days. Administration of 1,2,4,5-tetrachlorobenzene resulted in increased kidney and liver weights, increased mixed function oxidase activities, increased serum cholesterol values, moderate to marked histological liver changes in both sexes, and marked histological kidney changes in males. The authors concluded that 1,2,4,5-tetrachlorobenzene was the most toxic tetrachlorobenzene isomer when administered in the diet to rats, and that males appeared to be more susceptible than females. The authors did not specify the number or strain of animals used, if the effects observed were dose-related or only seen at the higher dose(s), the severeness of effects, or the type of histological changes observed.

Fomenko (1965) examined the subchronic toxicity of 1,2,4,5-tetrachlorobenzene in rats and rabbits. Both species of animals were given the compound daily by gavage in vegetable oil at dose levels of 0, 0.001, 0.005 or 0.05 mg/kg for 8 months. No treatment-related effects were observed in either rats or rabbits at the 0.001 mg/kg dose level. Doses of 0.005 or 0.05 mg/kg to rats induced a disruption of conditioned reflexes, increased liver weight coefficients, and decreased blood serum SH groups, while increased organ ascorbic acid was seen only in those rats given 0.05 mg/kg; the author did not indicate the statistical significance of these effects. Rabbits given 0.005 mg/kg had a transient disorder of liver glycogen formation and a statistically significant ($p=0.05$) change in blood serum SH groups during the last month of treatment. At the 0.05 mg/kg dose level, rabbits were observed to have a disorder of liver glycogen formation during the sixth month of treatment, increased serum blood SH groups in the fifth month that was followed by a decrease, a statistically significant ($p=0.05$) increase in the blood hemoglobin level during the third month of treatment, an increased level of peripheral reticulocytes at the end of the last month of treatment, and an increased retention of an intravenous galactose load by 6 months of treatment.

In a 2-month oral study, rats were given daily doses of 0 or 75 mg/kg 1,2,4,5-tetrachlorobenzene in vegetable oil by gavage (Fomenko, 1965). No treatment-related histologic changes were observed, but several biochemical parameters were affected, indicating changes in hepatic and hematopoietic homeostasis. The blood cholinesterase activity increased significantly ($p=0.01$), and the prothrombin index dropped by ~1/3 of the control value (p not reported). In addition, the number of peripheral reticulocytes decreased significantly ($p=0.02$) but then increased, the serum potassium levels were reduced (p not reported), and the number of peripheral large-diameter erythrocytes was increased (p not reported). The incidence of erythemia was significantly increased ($p=0.01$). At cessation of treatment, statistically significant ($p=0.01$) observed effects included a decrease in serum SH groups, adrenal hypertrophy and decreased adrenal ascorbic acid.

10.3.3. Chronic Toxicity. Braun et al. (1978) fed two beagle dogs of each sex diets containing doses of 5 mg/kg/day 1,2,4,5-tetrachlorobenzene for 2 years, and then observed them for a 20-month recovery period. The primary goal of the study was to determine the uptake and elimination kinetics for plasma and fat; therefore, no concurrent control animals were used. Historical control data, however, suggested that the elevations of serum alkaline phosphatase and total bilirubin after 24 months of administration were related to treatment. The elevated clinical chemistry values returned to the normal range of values for the historical controls at 3 months into the 20-month recovery period. Gross and histopathological examinations of tissues done after the recovery period did not reveal any treatment-related morphological changes in the animals. This study cannot substantiate either a no-observed-effect level (NOEL) or a lowest-observed-effect level (LOEL), because concurrent controls were not used, the number of treated animals used was small, and only one dose level was tested.

10.3.4. Mutagenicity. Kiraly et al. (1979) reviewed the chromosomal effects of 1,2,4,5-tetrachlorobenzene in Hungarian workers and concluded that 1,2,4,5-tetrachlorobenzene is mutagenic in occupationally-exposed humans. A more accurate conclusion from this data is that 1,2,4,5-tetrachlorobenzene is clastogenic in the exposed humans. This paper was discussed in detail in Section 10.2.

Paradi and Lovenyak (1981) reported that 1,2,4,5-tetrachlorobenezene did not induce an increased frequency of sex-linked recessive lethals in *Drosophila melanogaster* exposed by larval feeding at a dose less than the LC_{50} (actual dose not reported). Because only an abstract of the original paper was evaluated, rather than the paper itself, there was no information regarding the number of chromosomes assayed at each dose nor the doses used. This information is essential before any conclusions can be made as to whether or not 1,2,4,5-tetrachlorobenzene can induce sex-linked recessive lethal mutations in *Drosophila*.

1,2,3,5- and 1,2,4,5-tetrachlorobenzene were tested for mutagenicity by plate incorporation with *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 at five unspecified dose levels (Lawlor et al., 1979). Both isomers gave negative results in the reverse mutation assay either in the presence or absence of an S-9 metabolic activation system from rats pretreated with Aroclor 1254. Because these results were reported in an abstract, insufficient experimental detail was provided to permit a critical evaluation of the data.

10.3.5. Carcinogenicity. Pertinent data regarding the carcinogenicity of 1,2,3,4-, 1,2,3,5- and 1,2,4,5-tetrachlorobenzene were not located in the available literature.

10.3.6. Reproductive and Teratogenic Effects. As reported in an abstract, Ruddick et al. (1981) administered 1,2,3,4-, 1,2,3,5- and 1,2,4,5-tetrachlorobenzene via gavage (vehicle not reported) at dose levels of 0, 50, 100 or 200 mg/kg to pregnant rats (10/dose level) on days 6 through 15 of gestation. 1,2,4,5-Tetrachlorobenzene was the most toxic isomer, inducing lethality in 9 of 10 treated dams at the 200 mg/kg level. A dose-related accumulation of compound residue was seen in dams and offspring with all three isomers, but was greatest in those animals given 1,2,4,5-tetrachlorobenzene. Other toxicity effects observed in dams treated with 1,2,4,5-tetrachlorobenzene included organ weight changes and significantly elevated serum cholesterol, liver aminopyrine-N-demethylase and hepatic aniline hydroxylase levels; it was unclear from the abstract whether these changes were dose-related or occurred at a single dose level. 1,2,3,4-Tetrachlorobenzene also induced maternal toxicity, manifested in a significantly lowered platelet count at the 200 mg/kg level. Fetotoxicity, as indicated by increased lethality of pups, was observed at the 200 mg/kg level of 1,2,3,4- and 1,2,3,5-tetrachlorobenzene. One malformed pup and minor chondrogenic delay were seen among the offspring of dams given 1,2,3,5-tetrachlorobenzene.

10.4. INTERACTIONS

Tetrachlorobenzene is capable of inducing the NADPH-dependent cytochrome P-450 metabolizing enzymes, which are nonspecific for natural and xenobiotic substrates (Ariyoshi et al., 1974, 1975a,b). The substrate may either be detoxified by such metabolism or become more hazardous (toxified) if converted to a reactive intermediate capable of binding to critical intracellular macromolecules. In itself, P-450 induction is not a disadvantage, but it may become one when substrates are activated during metabolism (Neal, 1980). Thus, exposure to tetrachlorobenzene may enhance the toxicity of a compound that normally would be innocuous. No studies were available, however, to demonstrate the interaction of tetrachlorobenzene with other compounds.

10.5. SUMMARY

No animal studies on pharmacokinetics, acute toxicity, subchronic toxicity, chronic toxicity, mutagenicity, carcinogenicity or reproductive and teratogenic effects of 1,2,3,4-, 1,2,3,5- or 1,2,4,5-tetrachlorobenzene following inhalation exposure were located in the available literature.

Tetrachlorobenzenes are lipid-soluble compounds that bioaccumulate in the fat of aquatic and terrestrial organisms. Although the isomers were preferentially distributed to adipose tissue, they did not cross the blood-brain barrier of rabbits. Some Tokyo residents were found to have 1,2,4,5-tetrachlorobenzene (mean of 0.019 mg/kg bw) in their adipose tissue.

The metabolism of the tetrachlorobenzene isomers apparently follows aromatic hydroxylation to tetrachlorophenols with an arene oxide intermediate. Rabbits and rats treated with the tetrachlorobenzene isomers excreted unchanged compound in expired air and feces; the urine contained tetrachlorophenols, trichlorophenols, dihydroxylated tetrachlorobenzenes, glucuronides and ethereal sulfates.

The tetrachlorobenzenes have been reported as metabolites of lindane in rats, molds, hen pheasants, wheat, lettuce and endives, and of hexachlorobenzene in rats.

Chromosome aberrations were observed in the lymphocytes of Hungarian workers producing 1,2,4,5-tetrachlorobenzene; no airborne concentrations or exposures were determined.

The only mammalian toxicology data available for tetrachlorobenzenes are the result of oral exposures. The oral LD_{50} for 1,2,4,5-tetrachlorobenzene was reported as 1035 mg/kg in mice and 1500 mg/kg in rats and rabbits when administered in sunflower oil and 2650 mg/kg in mice when administered in 1.5% starch solution. Subchronic oral exposure of rats and rabbits to 1,2,4,5-tetrachlorobenzene resulted in statistically significant effects on biochemical parameters, including reticulocytosis, increased blood cholinesterase activity, erythremia and an indication that glycogen formation was impeded; at higher doses of 1,2,4,5-tetrachlorobenzene, rats also had increased kidney and liver weights, and renal and hepatic histologic changes.

Reversible effects on serum alkaline phosphatase and total bilirubin were reported in dogs given 5 mg/kg/day 1,2,4,5-tetrachlorobenzene in the diet for 2 years.

1,2,4,5-Tetrachlorobenzene was not mutagenic in the sex-linked recessive lethal assay with Drosophila melanogaster. However, because only an abstract of the Drosophila study was available, experimental details were too sparse to permit a critical evaluation of this negative result. Both 1,2,3,5- and 1,2,4,5-tetrachlorobenzene were negative in the reverse mutation assay with Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538. These results were reported in an abstract with insufficient experimental detail. Also, a negative result in the Salmonella assay with chlorinated compounds is not unexpected.

No information was available regarding the carcinogenicity of any of the three tetrachlorobenzene isomers in either animals or humans.

The tetrachlorobenzene isomers induced appreciable maternal toxicity, mild fetotoxicity and negligible teratogenicity in rats following oral administration.

United States
Environmental Protection
Agency

Office of Health and
Environmental Assessment
Washington DC 20460

EPA-600/8-84-015A
April 1984
External Review Draft

Research and Development

EPA

Health Assessment Document for Chlorinated Benzenes

Part 2 of 2

Review
Draft

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EPA-600/8-84-015A
April 1984
External Review Draft

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HEALTH ASSESSMENT DOCUMENT FOR CHLORINATED BENZENES

Part 2 of 2

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10-27

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PREFACE

The Office of Health and Environmental Assessment of the Office of Research and Development has prepared this Health Assessment Document (HAD) on chlorinated benzenes at the request of the Office of Air Quality, Planning and Standards. The chlorinated benzenes are a group of 12 chlorinated cyclic aromatic compounds which are currently being studied by the Environmental Protection Agency (EPA) to determine if they should be regulated as hazardous air pollutants under the Clean Air Act.

In the development of this assessment document, the scientific literature has been searched and inventoried, key studies have been reviewed and evaluated and summaries and conclusions have been directed at identifying the health effects from exposure to the various chlorinated benzenes. At several stages in the HAD development process, the chlorinated benzenes document has been reviewed for scientific and technical accuracy. These peer reviews have been by scientists from inside and outside the EPA. Observed effect levels and dose-response relationships are discussed where appropriate in order to identify the critical effect and to place adverse health responses in perspective with observed environmental levels.

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TABLE OF CONTENTS

Page

1. INTRODUCTION 1-1

2. SUMMARY AND CONCLUSIONS 2-1

  2.1. SUMMARY 2-1

    2.1.1. Properties, Production and Use. 2-1
    2.1.2. Environmental Levels, Transport and Fate. 2-2
    2.1.3. Ecological Effects. 2-4
    2.1.4. Pharmacokinetics. 2-5
    2.1.5. Effects on Humans 2-10
    2.1.6. Mammalian Toxicology. 2-12

  2.2. CONCLUSIONS 2-20
  2.3. NEEDS FOR FUTURE RESEARCH 2-20

3. PHYSICAL AND CHEMICAL PROPERTIES/ANALYTICAL METHODOLOGY 3-1

  3.1. SYNONYMS, TRADE NAMES AND IDENTIFICATION NUMBERS. 3-1
  3.2. PHYSICAL AND CHEMICAL PROPERTIES. 3-1
  3.3. ANALYTICAL METHODOLOGY. 3-15

    3.3.1. Chemical Analysis in Air. 3-15
    3.3.2. Chemical Analysis in Water. 3-18
    3.3.3. Chemical Analysis in Soil, Sediment and Chemical Waste Disposal Site Samples 3-19
    3.3.4. Chemical Analysis in Fish and Other Foods 3-20

  3.4. SUMMARY 3-23

4. PRODUCTION, USE AND ENVIRONMENTAL LEVELS. 4-1

  4.1. PRODUCTION. 4-1
  4.2. USE 4-5
  4.3. SOURCE AND ENVIRONMENTAL LEVELS 4-5

    4.3.1. Levels in Air 4-12
    4.3.2. Water 4-18
    4.3.3. Food. 4-26
    4.3.4. Soil and Sediments. 4-28
    4.3.5. Human Tissue Residues 4-29

  4.4. RELATIVE SOURCE CONTRIBUTIONS TO TOTAL EXPOSURE 4-34

    4.4.1. Air 4-36
    4.4.2. Water 4-36
    4.4.3. Food. 4-38

  4.5. SUMMARY 4-38

Page

5. ENVIRONMENTAL TRANSPORT AND FATE 5-1

  5.1. TRANSPORT 5-1

    5.1.1. Air 5-1
    5.1.2. Water 5-2
    5.1.3. Soil. 5-4

  5.2. FATE. 5-7

    5.2.1. Air 5-7
    5.2.2. Water 5-8
    5.2.3. Soil. 5-10

  5.3. BIOCONCENTRATION, BIOACCUMULATION AND BIOMAGNIFICATION. . . 5-13
  5.4. SUMMARY 5-19

6. ECOLOGICAL EFFECTS. 6-1

  6.1. EFFECTS ON THE AQUATIC ENVIRONMENT. 6-1

    6.1.1. Effect on Freshwater and Marine Fish. 6-1
    6.1.2. Effect on Aquatic Crustaceans 6-14
    6.1.3. Embryotoxic and Reproductive Effects. 6-16
    6.1.4. Effect on Aquatic Plants. 6-24
    6.1.5. Residues. 6-30

  6.2. EFFECTS ON NONAQUATIC ENVIRONMENTS. 6-34

    6.2.1. Plants. 6-34
    6.2.2. Insects 6-35
    6.2.3. Birds 6-37
    6.2.4. Residues. 6-38

  6.3. SUMMARY 6-39

7. MONOCHLOROBENZENE 7-1

  7.1. PHARMACOKINETICS. 7-1

    7.1.1. Absorption. 7-1
    7.1.2. Distribution. 7-1
    7.1.3. Metabolism. 7-2
    7.1.4. Excretion 7-5
    7.1.5. Summary 7-10

  7.2. EFFECTS ON HUMANS 7-10

Page

7.3. MAMMALIAN TOXICITY. 7-12

7.3.1. Acute Toxicity. 7-12
7.3.2. Subchronic Toxicity 7-15
7.3.3. Chronic Toxicity. 7-23
7.3.4. Mutagenicity. 7-24
7.3.5. Carcinogenicity 7-24
7.3.6. Reproductive and Teratogenic Toxicity 7-31

7.4. INTERACTIONS. 7-31
7.5. SUMMARY 7-32

8. DICHLOROBENZENES. 8-1

8.1. PHARMACOKINETICS. 8-1

8.1.1. Absorption. 8-1
8.1.2. Distribution. 8-3
8.1.3. Metabolism. 8-6
8.1.4. Excretion 8-8
8.1.5. Summary 8-9

8.2. EFFECTS ON HUMANS 8-10

8.2.1. Occupational Studies. 8-10
8.2.2. Case Studies. 8-11
8.2.3. Summary 8-17

8.3. MAMMALIAN TOXICOLOGY. 8-17

8.3.1. Acute Toxicity. 8-17
8.3.2. Subchronic Toxicity 8-22
8.3.3. Chronic Toxicity. 8-32
8.3.4. Mutagenicity. 8-33
8.3.5. Carcinogenicity 8-34
8.3.6. Reproductive and Teratogenic Toxicity 8-39

8.4. INTERACTIONS. 8-39
8.5. SUMMARY 8-40

9. TRICHLOROBENZENES 9-1

9.1. PHARMACOKINETICS. 9-1

9.1.1. Absorption. 9-1
9.1.2. Distribution. 9-2
9.1.3. Metabolism. 9-2
9.1.4. Excretion 9-6
9.1.5. Summary 9-8

9.2. EFFECTS IN HUMANS 9-9

Page

9.3. MAMMALIAN TOXICOLOGY. 9-9

- 9.3.1. Acute Toxicity. 9-9
- 9.3.2. Subchronic Toxicity 9-14
- 9.3.3. Chronic Toxicity. 9-23
- 9.3.4. Mutagenicity. 9-24
- 9.3.5. Carcinogenicity 9-25
- 9.3.6. Reproductive and Teratogenic Toxicity 9-26

9.4. INTERACTIONS. 9-28
9.5. SUMMARY 9-28

10. TETRACHLOROBENZENES 10-1

10.1. PHARMACOKINETICS. 10-1

- 10.1.1. Absorption. 10-1
- 10.1.2. Distribution. 10-2
- 10.1.3. Metabolism. 10-7
- 10.1.4. Excretion 10-10
- 10.1.5. Summary 10-13

10.2. EFFECTS ON HUMANS 10-14
10.3. MAMMALIAN TOXICOLOGY. 10-15

- 10.3.1. Acute Toxicity. 10-15
- 10.3.2. Subchronic Toxicity 10-20
- 10.3.3. Chronic Toxicity. 10-22
- 10.3.4. Mutagenicity. 10-22
- 10.3.5. Carcinogenicity 10-23
- 10.3.6. Reproductive and Teratogenic Effects. 10-23

10.4. INTERACTIONS. 10-24
10.5. SUMMARY 10-24

11. PENTACHLOROBENZENE. 11-1

11.1. PHARMACOKINETICS. 11-1

- 11.1.1. Absorption. 11-1
- 11.1.2. Distribution. 11-2
- 11.1.3. Metabolism. 11-7
- 11.1.4. Excretion 11-11
- 11.1.5. Summary 11-13

11.2. EFFECTS ON HUMANS 11-13

x
10-36

Page

11.3. MAMMALIAN TOXICOLOGY. 11-13

11.3.1. Acute Toxicity. 11-13
11.3.2. Subchronic Toxicity 11-16
11.3.3. Chronic Toxicity. 11-18
11.3.4. Mutagenicity. 11-19
11.3.5. Carcinogenicity 11-19
11.3.6. Reproductive and Teratogenic Toxicity 11-19

11.4. INTERACTIONS. 11-26
11.5. SUMMARY 11-26

12. HEXACHLOROBENZENE 12-1

12.1. PHARMACOKINETICS. 12-1

12.1.1. Absorption. 12-1
12.1.2. Distribution. 12-3
12.1.3. Metabolism. 12-16
12.1.4. Excretion 12-20
12.1.5. Summary 12-27

12.2. EFFECTS ON HUMANS 12-29

12.2.1. Epidemiologic Studies 12-29
12.2.2. Accidental Ingestion in Turkey. 12-32
12.2.3. Summary 12-37

12.3 MAMMALIAN TOXICOLOGY. 12-40

12.3.1. Acute Toxicity. 12-40
12.3.2. Subchronic Toxicity 12-42
12.3.3. Chronic Toxicity. 12-56
12.3.4. Mutagenicity. 12-59
12.3.5. Carcinogenicity 12-60
12.3.6. Reproductive and Teratogenic Effects. 12-125

12.4. INTERACTIONS. 12-131
12.5. SUMMARY 12-134

13. OVERVIEW OF EFFECTS OF MAJOR CONCERN. 13-1

13.1. PRINCIPAL EFFECTS AND TARGET ORGANS 13-1
13.2. ANIMAL TOXICITY STUDIES MOST USEFUL FOR HEALTH ASSESSMENT AND ESTIMATED TOXICITY THRESHOLDS. 13-5

13.2.1. Animal Toxicity Studies 13-5
13.2.2. Estimated Toxicity Thresholds 13-31

13.3. CARCINOGENICITY STUDIES 13-31
13.4. HUMAN STUDIES 13-39

Page

13.5. FACTORS INFLUENCING HEALTH HAZARD ASSESSMENT. 13-40

13.5.1. Exposure. 13-40

13.6. REGULATIONS AND STANDARDS 13-45

13.6.1. Occupational Standards. 13-45
13.6.2. Transportation Regulations. 13-51
13.6.3. Solid Waste Regulations 13-52
13.6.4. Food Tolerances 13-54
13.6.5. Water Regulations 13-54
13.6.6. Air Regulations 13-55

14. REFERENCES. 14-1

APPENDIX A: Comparison Among Different Extrapolation Models. A-1

LIST OF TABLES

No.	Title	Page
3-1	Synonyms, Trade Names and Identification Numbers of the Chlorinated Benzenes.	3-3
3-2	Physical Properties of the Chlorinated Benzenes	3-7
3-3	Vapor Pressures and Vapor Densities of the Chlorinated Benzenes.	3-8
3-4	Reported Composition of Commercial 1,2-Dichlorobenzene.	3-11
4-1	United States Production of Chlorinated Benzenes for Selected Years.	4-2
4-2	U.S. Producers and Estimated Annual Production Capacities (1983) of Chlorobenzenes.	4-6
4-3	A Summary of the Uses of the Chlorinated Benzenes	4-8
4-4	Estimated Quantities of Chlorobenzenes Lost During Manufacture, and to the Environment Compared with Total Production in 1983.	4-9
4-5	Estimated Quantities of Hexachlorobenzene (HCB) in Industrial Wastes and Byproducts in 1972.	4-11
4-6	Chlorinated Benzene Levels in Ambient Air from Different Locations in the U.S.	4-13
4-7	Concentrations of Chlorinated Benzenes at Three Sites	4-16
4-8	Overall and Site-Specific Mean Atmospheric Levels of Chlorobenzenes throughout the United States	4-17
4-9	Atmospheric Levels of Hexachlorobenzene Around Selected Industrial Plants	4-19
4-10	Chlorinated Benzenes in Surface Water	4-20
4-11	Chlorobenzene Concentrations in Drinking Water from Ontario, Canada	4-24
4-12	Frequency and Range of Concentrations of Chlorinated Benzenes Pollutants in Industrial Wastewaters	4-27
4-13	Chlorinated Benzene Residues in Human Adipose Tissue.	4-30
4-14	Chlorinated Benzenes in the Blood of Nine Residents of Love Canal in Niagara Falls, New York	4-33

No.	Title	Page
4-15	Chlorinated Benzenes in the Breath and Urine of Nine Residents of Love Canal in Niagara Falls, New York.	4-35
4-16	Estimated Yearly Exposure to Several Chlorinated Benzenes Via Inhalation.	4-37
5-1	Predicted Transport and Fate of Chlorinated Benzenes Released from Landfills and Lagoons	5-5
5-2	Transport of Chlorinated Benzenes in Sandy Soil	5-6
5-3	Estimated Atmospheric Residence Time and Daily Loss Rates for Several Chlorinated Benzenes.	5-9
5-4	Aqueous Biodegradability Studies of Chlorinated Benzenes. . .	5-11
5-5	Octanol/Water Partition Coefficients, Bioconcentration Factors and Biological Half-lives for Chlorinated Benzenes in Fish	5-15
5-6	Bioconcentration Factor and Slope of the Elimination Curve for Guppies (Poecilia reticulata) Exposed to Six Chlorinated Benzenes.	5-18
6-1	Acute Toxicity Data for Fish Species Exposed to Chlorinated Benzenes.	6-2
6-2	Chronic Toxicity Values of Chlorinated Benzenes in Fish . . .	6-12
6-3	Bioconcentration Factors of Some Chlorinated Benzenes in Two Fish Species	6-15
6-4	Acute Toxicity Data for Crustaceans Exposed to Chlorinated Benzenes.	6-17
6-5	Embryo-Larval Toxicity of Monochlorobenzene to Goldfish, Largemouth Bass and Rainbow Trout in Soft and Hard Water. . .	6-21
6-6	Results of 1,2,4,5-Tetrachlorobenzene Tests with Embryo to Juvenile Sheepshead Minnows in Continuous-Flow Natural Seawater.	6-23
6-7	Adult Lifespan and Reproductive Performance of Brine Shrimp Exposed to 1,3,5-Trichlorobenzene.	6-25
6-8	Acute Toxicity Data for Aquatic Algae Exposed to Chlorinated Benzenes.	6-26
6-9	Chlorinated Benzene Concentrations (μg/ℓ) in Water and Sediment.	6-31

No.	Title	Page
6-10	Chlorinated Benzene Concentrations in a Variety of Marine Species	6-32
6-11	Emergence of Adult Houseflies 8 Days Following Exposure of Pupae to "Saturation Concentration" of Dichlorobenzene Vapors.	6-36
6-12	Chlorinated Benzene Residues in Bird Eggs	6-40
7-1	Percentage of Isomers of Chlorophenol from Metabolism of Monochlorobenzene.	7-4
7-2	Species Variation in Urinary Metabolites of ^{14}C-Monochlorobenzene	7-9
7-3	Acute Toxicity of Monochlorobenzene	7-14
7-4	Summary of Subchronic Toxicity Studies on Monochlorobenzene .	7-16
7-5	Mutagenicity Testing of Monochlorobenzene	7-25
7-6	Nonneoplastic Lesions in F344 Rats Given Chlorobenzene by Gavage for 2 Years.	7-28
7-7	Statistical Comparisons of Liver Tumors in Male Rats Treated with Chlorobenzene and Vehicle Controls	7-29
8-1	Tissue Concentrations of 1,4-Dichlorobenzene in Adult Female CFY Rats	8-5
8-2	Chromosomal Alterations in Persons Accidentally Exposed to 1,2-Dichlorobenzene.	8-12
8-3	Case Reports Involving Dichlorobenzenes (DCB)	8-13
8-4	Acute Toxicity of 1,2-Dichlorobenzene	8-19
8-5	Acute Toxicity of 1,4-Dichlorobenzene	8-20
8-6	Subchronic Toxicity of 1,2-Dichlorobenzene.	8-23
8-7	Subchronic Toxicity of 1,4-Dichlorobenzene.	8-25
8-8	NTP Bioassay of 1,2-Dichlorobenzene Analysis of Primary Tumors in Male Rats: Adrenal Pheochromocytomas. . . .	8-36
9-1	Distribution of ^{14}C-Labeled 1,2,4-Trichlorobenzene in Rat Tissues after Oral Dosing with 181.5 mg/kg/day for 7 Days . .	9-3
9-2	Summary of Subchronic and Chronic Toxicity Studies on Trichlorobenzenes.	9-15

No. Title Page

10-1 Percentage of 1,2,4,5-Tetrachlorobenzene Steady-State Reached at Specific Times in Fat and Plasma of Dogs 10-4
10-2 Time Required to Reach Various Percentages of 1,2,4,5-Tetrachlorobenzene Steady-State in Fat and Plasma of Dogs . . 10-5
10-3 Unchanged Tetrachlorobenzene in Rabbit Tissues 6 Days After Oral Dosing 10-6
10-4 Urinary Metabolites of Tetrachlorobenzene Isomers in Rabbits 6 Days After Oral Dosing. 10-9
10-5 Summary of Excretion of the Isomeric Tetrachlorobenzenes as Metabolites or as Unchanged Compound in Rabbits Dosed Orally. 10-11
10-6 Excretion of Unchanged Tetrachlorobenzenes in the Expired Air of Rabbits After Oral Dosing. 10-12
10-7 Frequency of Chromatid-type Chromosome Aberrations in Peripheral Lymphocytes. 10-16
10-8 Frequency of Labile Chromosome-type Aberrations 10-17
10-9 Frequency of Stable Chromosome-type Aberrations 10-18
10-10 Summary of Toxicity Studies on Tetrachlorobenzenes. 10-19
11-1 Distribution of Pentachlorobenzene Residues in the Tissues of Maternal Rats after Oral Administration. 11-3
11-2 Distribution of Pentachlorobenzene Residues in the Tissues of Fetal Rats after Oral Administration to Dams . . . 11-4
11-3 Distribution of Pentachlorobenzene and/or Metabolites on the 40th Day in the Rhesus Monkey Following a Single Oral Dose of 0.5 mg/kg Body Weight 11-6
11-4 Distribution of Pentachlorobenzene in Chinchilla Doe Rabbits Expressed as a Percentage of Administered Dose. . . . 11-8
11-5 Percentage of Pentachlorobenzene and Its Metabolites Identified in Urine, Feces and Various Organs of Rhesus Monkeys Dosed 0.5 mg/kg Body Weight Pentachlorobenzene. . . . 11-9
11-6 Cumulative Urinary and Fecal Excretion of Pentachlorobenzene and Metabolites During 40 Days Following a Single Oral Dose of 0.5 mg/kg in Male and Female Rhesus Monkeys. 11-12
11-7 Acute Oral Toxicity of Pentachlorobenzene 11-15

No. Title Page

11-8 Summary of Subchronic, Reproductive and Teratogenic Toxicity Studies on Pentachlorobenzene. 11-17

11-9 Reproductive Effects in Litters of Female Rats Fed Diets Containing Pentachlorobenzene 11-21

11-10 Toxic Effects of Pentachlorobenzene on Reproduction in Rats Dosed on Each of Gestation Days 6-15 11-23

11-11 Skeletal and Soft-Tissue Abnormalities Observed in Rat Litters of Dams Treated with Pentachlorobenzene on Each of Gestation Days 6-15. 11-24

11-12 Fetal Wistar Rat Residues of Pentachlorobenzene 11-25

12-1 Storage and Excretion of ^{14}C-HCB Administered Orally in Arachis Oil in Rats. 12-4

12-2 Tissue Concentration (ppm) of ^{14}C-Hexachlorobenzene and Its Metabolites in Sprague-Dawley Rats. 12-6

12-3 Tissue Levels of HCB (ppm) in Adult Female Rhesus Monkeys . . 12-7

12-4 HCB Concentrations in Tissues of Male Beagles Receiving Single Intravenous Doses of 1 mg/kg bw in Olive Oil 12-9

12-5 Mean (±SE) Hexachlorobenzene Radioactivity (dpm/g) of Selected European Ferret Tissues. 12-14

12-6 Mean (±SE) HCB Radioactivity (dpm x 10^3) of European Ferret Kits 12-15

12-7 Concentrations of HCB and its Metabolites (mg/kg) in the Liver and Kidneys of Male and Female Rats 12-19

12-8 Hexachlorobenzene and Its Major Metabolites in the Excreta of Different Animal Species 12-21

12-9 Results of Blood and Urine Analysis in Men Employed in a Chlorinated Solvents Plant, 1974-1977 12-31

12-10 HCB Plasma Levels in Exposed Individuals and Controls 12-33

12-11 Clinical Signs and Symptoms in Humans 25 Years After Exposure to Low Levels in HCB in Turkey, 1955-1959. 12-36

12-12 Porphyrin Levels in Patients and Controls 12-38

12-13 Laboratory Test Results of Turkish Patients 12-39

12-14 Summary of Toxicity Studies on Hexachlorobenzene. 12-43

No.	Title	Page
12-15	Porphyrin Content and Uroporphyrinogen Decarboxylase Activity in the Liver Cytosol of Female Rats Pretreated with 100 mg/kg HCB Every Other Day for 6 Weeks	12-53
12-16	Tumor Incidence in Hamsters Given HCB in the Diet	12-62
12-17	HCB Levels in Tissues of Male Rats Following Administration of 8 mg/kg in Sunflower Oil for 19 Days	12-64
12-18	HCB Levels in Tissues of Male Rats Following Administration of ^{14}C-HCB in Arachis Oil	12-65
12-19	Effect of HCB on Hamsters: Liver Tumors and Other Liver Lesions	12-69
12-20	Liver Tumor Incidence in Mice Fed HCB	12-71
12-21	Tumor Data on Mice Fed HCB	12-72
12-22	Body Weights of Female Agus Rats Fed Hexachlorobenzene for 90 Weeks	12-76
12-23	Growth Rates for Female Agus Rats on a Diet Containing 100 ppm HCB	12-78
12-24	Dosage Levels in the Chronic Feeding Study of Hexachlorobenzene in Sprague-Dawley Rats	12-81
12-25	Liver and Kidney Tumors in Sprague-Dawley Rats Given Hexachlorobenzene in the Diet for up to 2 Years	12-82
12-26	Adrenal Tumors in Sprague-Dawley Rats Given Hexachlorobenzene in the Diet for up to 2 Years	12-84
12-27	Exposure Levels in the Chronic Feeding, 2-Generation Study of Hexachlorobenzene in Sprague-Dawley Rats	12-86
12-28	Tumors in Organs that Showed Statistical Differences from Control in F_1 Sprague-Dawley Rats Treated with Hexachlorobenzene	12-88
12-29	Parathyroid and Adrenal Pheochromocytomas in Sprague-Dawley Rats Maintained on Synthetic Diets of Varying Vitamin A Content and With or Without Hexachlorobenzene	12-89
12-30	Qualitative Comparison of Tumor Development in Rats Following Hexachlorobenzene Administration in Different Studies	12-92
12-31	Tumor Incidences in Male and Female Hamsters Given Hexachlorobenzene in Diet	12-107

10-44

No. Title Page

12-32 Incidence of Liver Cell Tumors in Male and Female Swiss Mice Given Hexachlorobenzene Diet 12-108

12-33 Liver and Kidney Tumor Incidence Rates in Male and Female Sprague-Dawley Rats Given Hexachlorobenzene in Diet. . 12-109

12-34 Incidence Rate of Adrenal Pheochromocytoma in Female Sprague-Dawley Rats (F_1 generation) in a 2-Generation Feeding Study 12-110

12-35 The Carcinogenic Potency of Hexachlorobenzene, Calculated on the Basis of 14 Data Sets, Using the Linearized Multistage Model. 12-113

12-36 Upper-Bound (Point) Estimation of Risk, Based on Hepatocellular Carcinoma in Female Rats 12-115

12-37 Relative Carcinogenic Potencies Among 54 Chemicals Evaluated by the Carcinogen Assessment Group as Suspect Human Carcinogens 12-119

12-38 Significantly Increased Incidence of Tumors in Animals Given Hexachlorobenzene in Diet 12-124

12-39 Analysis of the Excreta from Rats Administered Hexachlorobenzene After an Initial Treatment with Diethylstilboestrol. 12-132

13-1 Summary of Subchronic Toxicity Studies on Monochlorobenzene . 13-6

13-2 Subchronic Toxicity of 1,2-Dichlorobenzene. 13-9

13-3 Subchronic Toxicity of 1,4-Dichlorobenzene. 13-11

13-4 Summary of Subchronic and Chronic Toxicity Studies on Trichlorobenzenes 13-13

13-5 Summary of Toxicity Studies on Tetrachlorobenzenes. 13-15

13-6 Summary of Subchronic, Reproductive and Teratogenic Toxicity Studies on Pentachlorobenzene. 13-16

13-7 Summary of Toxicity Studies on Hexachlorobenzene. 13-17

13-8 Comparison of Toxic Effects of Chlorinated Benzene Isomers in Rats 13-22

13-9 Comparison of Toxic Effects of Chlorinated Benzene Isomers in Mice 13-24

13-10 Comparison of Toxic Effects of Chlorinated Benzene Isomers in Rabbits. 13-26

No.	Title	Page
13-11	Comparison of Toxic Effects of Chlorinated Benzene Isomers in Dogs	13-28
13-12	Comparison of Toxic Effects of Chlorinated Benzene Isomers in Monkeys	13-30
13-13	Toxicity Data for Threshold Estimates	13-32
13-14	Summary of Tumors Induced in Rodents by HCB	13-37
13-15	Comparison of Chemical and Physical Properties of Chlorinated Benzenes	13-41
13-16	Comparison of Chlorinated Benzenes BCF and Water Concentrations	13-42
13-17	Estimated Yearly Exposure to Several Chlorinated Benzenes Via Inhalation	13-44
13-18	Occupational Standards for Monochlorobenzene	13-46
13-19	Occupational Standards for 1,2-Dichlorobenzene	13-48
13-20	Occupational Standards for 1,4-Dichlorobenzene	13-49
13-21	The Chlorinated Benzenes as Constituents of Hazardous Wastes from Specific Sources	13-53
13-22	Ambient Water Quality Criteria for Chlorinated Benzenes--Aquatic Life	13-56
13-23	Ambient Water Quality Criteria for the Chlorinated Benzenes for the Protection of Human Health	13-57
13-24	Maximum Immission Concentration Standards for Monochlorobenzene	13-59

LIST OF FIGURES

No.	Title	Page
3-1	Chemical Structure of the Chlorinated Benzenes.	3-2
7-1	Metabolism of Monochlorobenzene	7-7
9-1	Metabolic Pathways for Trichlorobenzene (TCB) Isomers Through Arene Oxide Intermediates in Rabbits.	9-7
12-1	Histogram Representing the Frequency Distribution of the Potency Indices of 54 Suspect Carcinogens Evaluated by the Carcinogen Assessment Group.	12-118

11. PENTACHLOROBENZENE

The annual production of pentachlorobenzene in the United States was estimated to be 1-10 million pounds in 1977 (U.S. EPA, 1981a). Recent information indicates that the production and import of pentachlorobenzene into the U.S. is zero (U.S. EPA, 1983). The compound has been used as a pesticide, a chemical intermediate (Clement Associates, 1979; Ware and West, 1977) and as a flame retardant (Kwiatkowski et al., 1976). Pentachlorobenzene has been detected in surface waters (Barkley et al., 1980; Oliver and Nichol, 1982; Elder et al., 1981), drinking water contaminated by a toxic waste site (Barkley et al., 1981), aquatic sediments (Elder et al., 1981), fish and shellfish (Oliver and Nichol, 1982; Ten Berge and Hillebrand, 1974) and in some edibles (U.S. EPA, 1980a).

11.1. PHARMACOKINETICS

11.1.1. Absorption. Pentachlorobenzene has lipophilic characteristics and is therefore likely to be capable of crossing biological membranes. Several studies were available on the absorption of pentachlorobenzene after oral administration. One study discussed absorption after dermal application; however, no studies were available on absorption via the inhalation route. No studies were encountered on the distribution of pentachlorobenzene after inhalation or dermal exposure.

Parke and Williams (1960) studied the absorption and metabolic fate of pentachlorobenzene in rabbits. Three to four days after a 0.5 g/kg dose of pentachlorobenzene suspended in an aqueous solution was administered by gavage, 5% was recovered in the feces and 45% was found in the gut contents. Biliary excretion was not measured; therefore, some of the pentachlorobenzene found in the gut and feces may have resulted from a portion of the dose that was absorbed being excreted unchanged in the bile. Rozman et al. (1979) found that absorption in two male and two female rhesus monkeys was very efficient. Four days following a single dose of 0.5 mg/kg pentachlorobenzene by gavage, at least 95% was reported as being absorbed. Blood and tissue levels of pentachlorobenzene and/or its metabolites were found to be similar to those measured for hexachlorobenzene, indicating the involvement of the lymphatic system in the absorption process (Iatropoulos et al., 1975). Other studies concerning the toxicity and metabolism of pentachlorobenzene (Linder et al., 1980; Engst et al., 1976; Villeneuve and Khera, 1975) also demonstrated that absorption occurs through the gastrointestinal tract, but did not provide quantitative data.

In the only available study involving dermal absorption of pentachlorobenzene, Linder et al. (1980) applied a single dose of 2500 mg/kg pentachlorobenzene dissolved in xylene to the shaved backs and shoulder areas of two rats. No clinical signs of toxicity were observed in males or females, suggesting that percutaneous absorption of pentachlorobenzene was poor.

11.1.2. Distribution. Villeneuve and Khera (1975) studied the distribution of pentachlorobenzene in dams and fetuses after daily administration by gavage of pentachlorobenzene prepared in corn oil at levels of 40, 100 and 200 mg/kg to pregnant rats on days 6-15 of gestation. On day 22, the dams were killed, fetuses removed and tissues analyzed by gas-liquid chromatography for organohalogen residues. Recovery of pentachlorobenzene was >80% for all tissues. In the tissues of the maternal animals, fat had the greatest accumulation of pentachlorobenzene, followed by the liver, brain, heart, kidneys and spleen. In the fetuses, the levels detected in the brain were equal to those measured in the whole fetus, while the levels in the liver were double the whole fetus concentration. Tables 11-1 and 11-2 show these distribution data. Both the maternal tissues and the whole fetuses appeared to accumulate pentachlorobenzene in a dose-related manner.

TABLE 11-1

Distribution of Pentachlorobenzene Residues in the Tissues of Maternal Rats after Oral Administration[a]

Dose Level (mg/kg)	Residue Concentration (mg/kg in wet tissue)[b]					
	Fat	Liver	Brain	Heart	Kidney	Spleen
50	470±106	13.9±5.1	6.9± 1.2	6.2±1.0	6.0±1.1	4.5±1.1
100	824±116	18.1±2.0	12.0± 1.7	12.6±2.0	10.6±1.5	8.3±1.3
200	3350±331	91.1±6.6	62.5±10.2	57.5±9.6	43.5±2.6	46.2±8.1

[a] Source: Villeneuve and Khera, 1975

[b] Represents the mean of five animals ± standard error of the mean

TABLE 11-2

Distribution of Pentachlorobenzene Residues in the Tissues of Fetal Rats after Oral Administration to Dams[a]

Dose Level (mg/kg)	Whole Fetus[b] (mg/kg)	Whole Fetus[b] (total μg)	Liver[c] (mg/kg)	Brain[c] (mg/kg)
50	2.44±0.38	9.65±1.3	4.37±0.69	3.08±0.55
100	5.27±0.60	21.2 ±2.1	10.4 ±1.31	5.31±0.60
200	16.9 ±2.8	55.1 ±6.7	40.4 ±6.02	20.5 ±2.64

[a] Source: Villeneuve and Khera, 1975

[b] Represents the mean of two fetuses each from 15 litters ± standard error of the mean.

[c] Represents the mean of five fetuses each from a different litter ± standard error of the mean.

Linder et al. (1980) also reported that pentachlorobenzene accumulated in the adipose tissue. Based on food consumption data provided by the authors, groups of 10 male rats were fed 6-16 or 50-134 mg/kg/day (125 or 1000 ppm) for 100 days, and similar groups of females were fed 6-16, 16-31, 27-63 or 55-134 mg/kg/day (125, 250, 500 or 1000 ppm) for 180 days. The results indicated that pentachlorobenzene accumulated in adipose tissue ~1.5-2.2 times the dietary concentration, and the accumulation was dose-dependent. Residues in males and females were similar, but could not be compared directly because of the longer exposure period of the females and the complicating factors of pregnancy and lactation. Suckling pups whose mothers were fed ≥250 ppm pentachlorobenzene developed tremors, and at 1000 ppm, most died before weaning. Though no clinical signs of tremors were observed in the parents, the authors stated that this result was presumptive evidence for excretion of a toxic agent via the milk. Because pentachlorobenzene accumulates in the fetus (Villeneuve and Khera, 1975), prenatal exposure of the pups may also have contributed to the observed effects.

Rozman et al. (1979) studied the distribution of pentachlorobenzene and its metabolites in four rhesus monkeys. Tissues of monkeys given a single dose of ^{14}C-labeled pentachlorobenzene (0.5 mg/kg) by gavage were analyzed after 40 days. Quantitative determination of pentachlorobenzene and its metabolites was performed by gas chromatography. The highest concentrations were found in the fat and bone marrow, followed by the thymus, lymph nodes and adrenal cortex. Table 11-3 summarizes the distribution data for the 20 tissues examined.

Parke and Williams (1960) studied the distribution of pentachlorobenzene in rabbits and found that the compound was readily isolated from the feces and gut contents 3-4 days following administration by gavage of 0.5 g/kg.

TABLE 11-3

Distribution of Pentachlorobenzene and/or Metabolites on the 40th Day in the Rhesus Monkey Following a Single Oral Dose of 0.5 mg/kg Body Weight[a]

Organ	Male (mg/kg)	Female (mg/kg)
Fat[b]	1.86	2.68
Bone marrow	1.10	2.35
Lymph nodes[b]	0.35	0.79
Thymus	0.50	0.61
Adrenal cortex	0.31	0.56
Adrenal medulla	0.18	0.07
Skin	0.26	0.26
Kidneys	0.09	0.10
Liver	0.19	0.17
Lungs	0.06	0.06
Spleen	0.04	0.04
Heart	0.07	0.12
Bile	0.09	0.09
Stomach	0.06	0.06
Duodenum	0.11	0.06
Cecum	0.24	0.18
Large intestine	0.31	0.33
Small intestine	0.17	0.07
Brain	0.05	0.06
Cerebellum	0.05	0.06

[a] Source: Rozman et al., 1979

[b] Average value from five different parts of the body

Subcutaneous injections of 0.5 g/kg (10% w/v solutions in arachis oil) resulted in concentrations of 47% in the pelt (mostly at the site of injection), 22% in the fat, a total of 2% in the gut and feces, and 10% in the rest of the body. Table 11-4 summarizes the distribution data for pentachlorobenzene for this study.

11.1.3. Metabolism. The metabolism of pentachlorobenzene has been studied in male Wistar rats by Engst et al. (1976) following administration by gavage of 8 mg/kg pentachlorobenzene dissolved in 1 mℓ of filtered sunflower oil. The major metabolites detected in the urine were identified as 2,3,4,5-tetrachlorophenol and pentachlorophenol. Pentachlorobenzene, 2,3,4,6-tetrachlorophenol and/or 2,3,5,6-tetrachlorophenol were present in the free form. Trichlorophenol (isomer not specified), 2,4,6-trichlorophenol and 1,2,3,4-tetrachlorobenzene were present in small concentrations. Quantities of the metabolites obtained were not reported for this study.

Koss and Koransky (1977) reported pentachlorophenol, 2,3,4,5-tetrachlorophenol, tetrachlorohydroquionone and a hydroxylated chlorothio compound as metabolites of pentachlorobenzene in the urine and feces of three female rats collected for 4 days after administering a single intraperitoneal dose of 403 μM/kg (sic). Pentachlorophenol and other hydrophilic metabolites accounted for 9% of the eliminated dose.

Rozman et al. (1979) measured and identified the metabolites of pentachlorobenzene in the rhesus monkey. Table 11-5 summarizes the metabolic breakdown during 40 days following a single oral dose by gavage of 0.5 mg/kg ^{14}C-labeled pentachlorobenzene. The major metabolites identified in the urine were pentachlorophenol, 2,3,4,5-tetrachlorophenol and 2,3,5,6-tetrachlorophenol. No significant differences were observed in the metabolism patterns of male and female monkeys.

TABLE 11-4

Distribution of Pentachlorobenzene in Chinchilla Doe Rabbits Expressed as a Percentage of Administered Dose[a]

Dose/Route (g/kg)	Time After Dosing (days)	Urine Tri- or Penta-chlorophenol	Other Phenols	Feces	Gut Contents	Pelt	Depot Fat	Rest of Body	Expired Air		Total Accounted %
									Unchanged	Other Chloro-hydrocarbons	
0.5 oral	3	0.2	1	5.0	45.0	1.0	15.0	6.0	0	9.0	82
0.5 oral	4	0.2	1	5.0	31.0	5.0	9.0	5.5	0	21.0	78
0.5 s.c.	10	0.7	1	1.5	0.5	47.0[b]	22.0[b]	10.0	0	<2.0	85

[a] Source: Parke and Williams, 1960

[b] Located mainly at site of injection

s.c. = subcutaneous

TABLE 11-5

Percentage of Pentachlorobenzene and Its Metabolites Identified in Urine, Feces and Various Organs of Rhesus Monkeys Dosed 0.5 mg/kg Body Weight Pentachlorobenzene*

	Pentachlorobenzene	1,2,3,4-Tetrachlorobenzene	Pentachlorophenol	2,3,4,5-Tetrachlorophenol	2,3,5,6-Tetrachlorophenol
Liver	99.0%	1.0%	ND	ND	ND
Bile	nonpolar compound(s)	nonpolar compound(s)	ND	ND	ND
Feces	99.0%	1.0%	ND	ND	ND
Blood	45.8%	ND	54.2%	ND	ND
Kidney	51.3%	ND	(- - - - - - - - - - 48.7% polar compound(s) - - - - - - - - - -)		
Urine	ND	ND	58.1%	32.2%	9.7%

*Source: Rozman et al., 1979

ND = Not detected

Similar results were obtained by Kohli et al. (1976) in male rabbits. Following intraperitoneal injection of 300 mg pentachlorobenzene dissolved in 10-15 mℓ vegetable oil, urinary metabolites were identified as 2,3,4,5-tetrachlorophenol and pentachlorophenol. Both were detected at yields of 1% during the 10 days following administration of the dose. Parke and Williams (1959) reported that <0.2% of the dose recovered in rabbit urine was pentachlorophenol.

The metabolic pathway of pentachlorobenzene was thought to involve oxidation and formation of an arene oxide intermediate by hepatic metabolic enzymes (Kohli et al., 1976). Subchronic feeding of 0.05% pentachlorobenzene in the diets of female adult Wistar rats for 60 days induced hepatic cytochrome P-450 content and enhanced the O-dealkylation of 7-ethoxycoumarin (Goerz et al., 1978), suggesting the involvement of the hepatic cytochrome P-450 system in metabolism. However, Rozman et al. (1979) reported that more phenolic intermediates were present in the blood, kidney and urine of monkeys than in the liver, bile and feces 40 days after a single dose of pentachlorobenzene. The evidence suggested that a metabolizing system other than hepatic cytochrome P-450 was involved in the hydroxylation of chlorinated benzenes. The authors proposed that two different hydroxylation pathways could be involved, one involving the oxidation of the pentachlorobenzene to pentachlorophenol, and the other involving the nucleophilic displacement reactions of pentachlorobenzene to tetrachlorophenols.

Koss and Koransky (1977) suggested that a major consideration in the toxicity of pentachlorobenzene is its metabolic transformation to pentachlorophenol. As previously stated, pentachlorophenol has been identified as a metabolite in the urine and excreta (Engst, 1976; Rozman et al., 1979;

Kohli et al., 1976; Parke and Williams, 1960). Rozman et al. (1979) demonstrated that the half-life of pentachlorobenzene in the rhesus monkey was 2-3 months, and after 40 days pentachlorophenol accounted for 58.1% of the metabolites identified in the urine.

11.1.4. **Excretion.** The excretion of pentachlorobenzene and its metabolites was described in rhesus monkeys following administration by gavage of a single oral dose of 0.5 mg/kg (Rozman et al., 1979). Approximately 12% of the administered dose was excreted in the urine after 40 days (see Table 11-5). After the same period, ~24% of the dose was excreted via the feces, of which 99% was unmetabolized. Table 11-6 displays the cumulative urinary and fecal excretion of pentachlorobenzene and its metabolites. This study indicated that the metabolites of pentachlorobenzene were excreted primarily via the urine, while the unabsorbed or unmetabolized compound was excreted via the feces. These results also indicated that pentachlorobenzene was eliminated very slowly, with an estimated excretion half-life in primates of 2-3 months.

Koss and Koransky (1977) identified 3% pentachlorobenzene in its unchanged form, pentachlorophenol, 2,3,4,5-tetrachlorophenol and a hydroxylated chlorothio compound in the feces of rats 4 days after intraperitoneal administration of 403 μM/kg (sic) pentachlorobenzene. Parke and Williams (1960) also isolated 5% pentachlorobenzene after 4 days from the feces of rabbits given 0.5 g/kg pentachlorobenzene orally.

Linder et al. (1980) fed pentachlorobenzene (250-1000 ppm) to female Sherman rats with suckling pups and observed that the pups developed tremors and most died before weaning in the 1000 ppm group. This work provides presumptive evidence for excretion of pentachlorobenzene via the milk.

TABLE 11-6

Cumulative Urinary and Fecal Excretion of Pentachlorobenzene and Metabolites During 40 Days Following a Single Oral Dose of 0.5 mg/kg in Male and Female Rhesus Monkeys[a,b]

	Days After Exposure					
	4	10	20	30	40	% Total Rocovered
Males						
urine	1.9	4.8	8.6	11.3	13.2	40.2
feces	6.3	11.5	19.3	23.6	27.0	
Females						
urine	2.4	4.3	7.8	10.0	11.4	33.2
feces	4.4	8.3	16.4	19.8	21.8	

[a] Source: Rozman et al., 1979

[b] Expressed in percent of the total administered dose

11.1.5. Summary. Although studies of the absorption of pentachlorobenzene indicated that absorption does occur through the gastrointestinal tract, the extent of absorption has not been determined. A study in rabbits indicated that up to 50% of a dose was absorbed within 3-4 days. Oral administration to monkeys indicated 95% absorption within 4 days. Absorption resulting from inhalation has not been studied, and absorption from dermal exposure was found to be rather poor in rats. Once absorbed, pentachlorobenzene is widely distributed to many tissues, with the highest levels appearing in fat, liver and bone marrow. A study in rats demonstrated that transport across placental membranes occurred readily and that accumulation of pentachlorobenzene in the fetus is highest in the liver. No studies were encountered that described the distribution of pentachlorobenzene after inhalation or dermal exposure.

The metabolism of pentachlorobenzene is not fully understood, but some studies suggested that metabolic activity other than the hepatic cytochrome P-450, xenobiotic metabolizing system may be involved. Metabolism appeared to be primarily via oxidation to two major metabolites, pentachlorophenol and 2,3,4,5-tetrachlorophenol, which were excreted in the urine. Metabolism and excretion occurred at a slow rate; an estimated elimination half-life for a single dose in primates was 2-3 months.

11.2. EFFECTS ON HUMANS

No epidemiologic studies or case studies of effects in humans resulting from exposure to pentachlorobenzene were available for review.

11.3. MAMMALIAN TOXICOLOGY

11.3.1. Acute Toxicity. Linder et al. (1980) investigated the acute and subchronic toxicity of 99.1% pure pentachlorobenzene in adult and weanling Sherman strain rats and adult Swiss-Webster mice. Weanling rats (27-35 days of age; 10 animals/dosage level) and adult animals (90-120 days of age; 10 animals/dosage level) were administered by gavage a single dose of 5.0-15.0 mℓ/kg pentachlorobenzene dissolved in peanut oil. The oral LD_{50} values ranged from 1080-1125 mg/kg for adult rats, and 1175-1370 mg/kg for adult mice; for weanling rats the LD_{50} was reported as 940 mg/kg (Table 11-7).

The characteristic toxic signs observed included a decrease in activity, hypersensitivity to touch, and tremors. The tremors started in mice ~24 hours after dosing and ~48 hours after dosing in rats. Death usually occurred in rats 5-12 days after dosing; in mice the survival time was less, with death usually occurring 2-4 days after the lethal dose was administered. The authors reported many cases of rats with reddish stains around the eyes, nose and mouth; no explanation of this phenomenon was given.

Ariyoshi et al. (1975) investigated the effects of various chlorinated benzenes, including pentachlorobenzene, on the microsomal drug metabolizing enzymes, δ-aminolevulinic acid synthetase, microsomal proteins and cytochrome P-450 content. Groups of 2-6 female Wistar rats were orally administered 250 mg/kg pentachlorobenzene suspended in a 2% tragacanth gum solution, once a day for 3 days. The compound increased the liver content of cytochrome P-450 and increased the activities of aniline hydroxylase and aminopyrine demethylase. Significant increases were also observed for microsomal protein and δ-aminolevulinic acid synthetase. Glycogen content decreased markedly, and triglyceride content increased in pentachlorobenzene-treated rats.

In the only available study involving acute dermal toxicity of pentachlorobenzene, Linder et al. (1980) applied a single dose of 2500 mg/kg pentachlorobenzene dissolved in xylene to the shaved backs and shoulder areas of two rats. No clinical signs of toxicity were observed in either male or female adult rats.

TABLE 11-7

Acute Oral Toxicity of Pentachlorobenzene*

Species/ Sex	Age	LD_{50} (mg/kg)	95% Confidence Limits (mg/kg)	Dosage Range Tested (mg/kg)	Dose Volume (mℓ/kg)
Rat/M	adult	1125	1015-1247	750-1350	7.5
Rat/F	adult	1080	952-1226	750-1500	7.5
Rat/F	weanling	940	864-1023	600-1200	5.0
Mouse/M	adult	1175	1035-1334	750-1500	15.0
Mouse/F	adult	1370	1263-1487	1050-1500	15.0

*Source: Linder et al., 1980

Weanling animals were 27-35 days old. Adult animals were 90-120 days old. Ten animals per each dosage group were given pentachlorobenzene dissolved in peanut oil.

11.3.2. Subchronic Toxicity. No studies of toxicity resulting from subchronic inhalatory exposure to pentachlorobenzene were located in the available literature. A summary of subchronic, reproductive and teratogenic toxicity studies on pentachlorobenzene can be found in Table 11-8.

Linder et al. (1980) studied the subchronic toxicity of pentachlorobenzene in rats as part of an investigation of the compound's toxic effects on reproduction. Groups of 10 female weanling rats were fed diets containing 0, 125, 250, 500 and 1000 ppm of pentachlorobenzene for ~180 days; while groups of 10 male rats received 0, 125 and 1000 ppm for 100 days. Based on food consumption data provided by the authors, it was estimated that the female groups consumed an average of 11, 23, 46 and 99 mg/kg/day, respectively (actual reported ranges of 7-16, 16-31, 27-63 and 55-134 mg/kg/day). The male groups consumed ~11 and 97 mg/kg/day, respectively (reported ranges of 7-16 and 50-134 mg/kg/day). None of the animals died or exhibited clinical signs of toxicity throughout the study. Food consumption and body weight gain for the dosed groups were similar to the control groups. In hematologic parameters, erythrocyte count and hematocrit were slightly lower than the control group ($p<0.05$) for the 1000 ppm males, and hemoglobin was reduced and leukocyte count increased in both 1000 ppm groups ($p<0.05$).

Examination of the liver and viscera under ultraviolet light did not reveal the presence of porphyrins in males or females. Only tissues of the female rats were analyzed quantitatively for porphyrins. Total liver porphyrins were slightly higher in female rats fed 1000 ppm compared with the control group (0.79 μg/g compared with 0.64 μg/g), but the difference was not judged to be a porphyrogenic response and was of doubtful consequence.

TABLE 11-8

Summary of Subchronic, Reproductive and Teratogenic Toxicity Studies on Pentachlorobenzene

Species	Route	Dose	Duration	Effects	Reference
Rat (female)	oral (diet)	125, 250, 500 or 1000 mg/kg in diet	180 days	Changes in hematologic parameters in high-dose group; increase in liver weights, hepatic hypertrophy and vacuolization in 500 and 1000 mg/kg groups; increased kidney weight in high-dose group	Linder et al., 1980
Rat (male)	oral (diet)	125 or 1000 mg/kg in diet	100 days	High-dose group induced changes in hematologic parameters; hepatic and renal histology and increase in liver, kidney and adrenal weights	Linder et al., 1980
Rat (offspring)	oral (diet)	125, 250, 500 or 1000 mg/kg in mothers diet	gestation and during suckling	Offspring treated with ≥ 250 mg/kg/diet were adversely affected (reduced survival, body weights and increased liver weights, hepatocellular enlargement)	Linder et al., 1980
Mice	oral	50 or 100 mg/kg/gavage	days 6-15 of gestation	Increase in liver weights of dams; no adverse effects on total development or survival	Courtney et al., 1979
Rat	oral	50, 100 or 200 mg/kg/gavage	days 6-15 of gestation	No observed toxicity in adult rats; increased total deaths at all doses, but not in dose-related manner; extra ribs in exposed fetuses and sternal defects in 200 mg/kg group	Khera and Villeneuve, 1975

Tissues of 21-day-old weanlings appeared normal although relative weights (organ/body weight ratio) of the livers were increased in pups of mothers fed $\geq$250 ppm for 180 days. The obvious change was hepatocellular enlargement in all pups from the groups fed 500 and 1000 ppm.

At necropsy of the adult rats, no pathological changes were observed in tissues of males fed pentachlorobenzene for 100 days or females fed pentachlorobenzene for 180 days. Weights of livers relative to body weight increased 35-45% in the animals fed 500 or 1000 ppm. Relative weights of the kidneys of both sexes and the adrenals of males increased in the 1000 ppm groups. Microscopically, hepatic cell enlargement (hypertrophy) and vacuolization were observed in the female rats of the 500 and 1000 ppm groups. Similar changes were apparent in the males fed 1000 ppm. In high-dose groups, the kidneys of males showed hyaline droplet formation, atrophic tubules and lymphocytic infiltration. Results of this study indicated that the toxicity of orally administered pentachlorobenzene was directed toward the liver and kidneys.

The ability of pentachlorobenzene to induce porphyria in Wistar rats has been investigated by Goerz et al. (1978). Adult female rats were fed a diet containing 0.05% (~25.0 mg/kg/day or 500 ppm) pentachlorobenzene for 60 days. This treatment increased the hepatic cytochrome P-450 content (1.06±0.30 and 1.20±0.30 nMol/mg microsomal protein for 10 and 60-day exposures, respectively, for the controls compared with 2.25±1.10 and 2.06±0.65 nMol/mg microsomal protein, for 10 and 60-day exposures for the pentachlorobenzene-treated rats), but did not increase the excretion of porphyrins in the urine.

11.3.3. Chronic Toxicity. No studies of toxicity resulting from chronic exposure of pentachlorobenzene were located in the available literature.

11.3.4. Mutagenicity. The only information available on the mutagenicity of pentachlorobenzene was a study presented in abstract form on a plate incorporation assay for reverse mutation in histidine-dependent strains of *Salmonella typhimurium* (Lawlor et al., 1979). Five strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537 and TA1538) were tested at five unspecified concentrations of pentachlorobenzene in the presence and absence of rat liver microsomes induced by Aroclor 1254. No detectable levels of mutagenic activity were found in the *Salmonella* tester strains (Lawlor et al., 1979). Because these results were reported in an abstract, experimental details were too sparse to permit a critical evaluation of this negative result. Also, this result is not unexpected because the *Salmonella* test system is generally insensitive to chlorinated compounds.

11.3.5. Carcinogenicity. The Ambient Water Quality Criteria Document for Chlorinated Benzenes (U.S. EPA, 1980b) cited a study by Preussman (1975) that was reported as alluding to the carcinogenicity of pentachlorobenzene. The German text is now being translated and reviewed by the Carcinogen Assessment Group of the U.S. EPA.

11.3.6. Reproductive and Teratogenic Toxicity. The reproductive toxicity of pentachlorobenzene was demonstrated in three studies. Of these studies, Linder et al. (1980) and Khera and Villeneuve (1975) provided sufficient data to estimate a NOEL and a LOAEL, respectively.

Linder et al. (1980) tested 99.1% pure pentachlorobenzene for its toxic effects on reproduction in rats. No other chlorinated compounds were detected by GC-EC analysis of the sample. Dietary concentrations of 0 (control), 125, 250, 500 and 1000 ppm (7.4-16, 16-31, 27-63 and 55-134 mg/kg/day) were fed by gavage to groups of ten 4- to 5-week-old weanling female Sherman strain rats, while similar groups of males received diets containing 0 (control), 125 or 1000 ppm (6-16 or 50-134 mg/kg/day). Both males and females were fed treated diets for 67 days before mating with untreated males or females. Pregnant females continued to receive treated diets until their litters were weaned, for a total exposure of 180 days; males were dosed for a total of 100 days before being sacrificed.

Litters sired by treated males showed no treatment-related effects. Although clinical signs were not observed in the parents, litters from treated females (≥250 ppm) were adversely affected. Pup survival and body weight at weaning were reduced in the two highest dose groups, and the offspring of the 250, 500 and 1000 ppm groups showed statistically significant ($p<0.05$) increases in liver-to-body weight ratios. Survival decreased dramatically from 88.6 to 28.0% during days 4-21 for pups whose mothers were fed concentrations of 500 and 1000 ppm, respectively. Table 11-9 summarizes the reproductive effects in litters of treated females.

Histologic examination of the livers of weanling rats revealed hepatocellular enlargement in all pups examined from the 500 and 1000 ppm groups, and in 2 of 9 male pups from the 250 ppm group. The hepatotoxic effects were not seen in the offspring of the dams exposed to dietary concentrations of 125 ppm. These data indicated that pentachlorobenzene was transferred to the offspring during gestation and/or lactation and had a toxic effect on the pups in the 250, 500 and 1000 ppm groups. Therefore, this study suggested a NOEL of 125 ppm in the diet for no toxic effects on the reproduction of rats.

In a study by Khera and Villeneuve (1975), pregnant Wistar rats (17-19/group) were administered pentachlorobenzene by gavage on days 6-15 of gestation. The doses in mg/kg with percentage concentrations in corn oil (in parentheses) were 50 (0.5), 100 (1.0) or 200 (2.0). Uterine and viscera

TABLE 11-9

Reproductive Effects in Litters of Female Rats Fed Diets Containing Pentachlorobenzene[a]

Parameter		Pentachlorobenzene in Diet (ppm)				
		0	125	250	500	1000
Dosage range (mg/kg/day)		NA	6-16	16-31	27-63	50-134
Litters born		8	6	9	9	8
Pups per litter (mean)		10.4	12.0	11.9	13.2	10.8
Litters weaned		8	6	9	9	4
Pup survival (%)						
Days 0-4		100	98.6	98.1	98.3	94.2
Days 4-21		100	91.7	95.4	88.6	28.0
Pup body weight at weaning[b]	Male	45 (5)	44 (3)	41 (4)	40 (4)	31 (4)
	Female	45 (8)	41 (3)	40 (3)	38 (4)	37 (4)
Liver/body weight ratio[c] (g/100 g)	Male	3.9 (0.1)	3.9 (0.1)	4.3 (0.1)[d]	5.1 (0.1)[d]	6.5 (0.1)[d]
	Female	4.0 (0.1)	3.9 (0.1)	4.2 (0.1)	5.3 (0.1)[d]	6.5 (0.2)[d]

[a] Source: Linder et al., 1980

[b] Values are litter means in grams (± standard deviation)

[c] Values are group means (± standard error of the mean)

[d] Significantly different from control; $p=0.05$ (statistical analysis performed on liver weights only)

NA = Not applicable

contents were removed following sacrifice of the dams on day 22 of gestation. No overt signs of toxicity were observed in the adult rats; however, the treatment increased fetal death rate at all of the doses tested, but not in a dose-related manner (Table 11-10). This study demonstrated a lethal effect of in utero exposure to pentachlorobenzene at doses to the dams as low as 50 mg/kg/day, therefore identifying a LOAEL for this study.

Khera and Villeneuve (1975) also reported that sternal defects were observed in the fetuses of Wistar rat mothers treated with 200 mg/kg/day. In addition, all three doses increased the incidence of both uni- and bilateral extra ribs (Table 11-11). The latter effect (increase in extra ribs), although not a gross malformation, occurred at an incidence 5-9 times greater than the controls, indicating a potential for pentachlorobenzene to alter fetal skeletal development. In addition, quantitative chemical analysis of fetuses for pentachlorobenzene residues showed a dose-related accumulation of the unchanged compound in the whole fetus, brain and liver (Table 11-12) (Villeneuve and Khera, 1975). These results suggested that the parent compound may have been responsible for the observed teratogenic and reproductive effects, but did not preclude metabolites as potential causes of the observed effects.

In a study of possible reproductive and teratogenic effects, Courtney et al. (1977) reported that no reproductive toxicity occurred in litters of pregnant CD-1 mice treated by gavage with 50 or 100 mg/kg of >97% pure pentachlorobenzene in 0.1 mℓ corn oil on days 6-15 of gestation. There were no teratogenic effects observed in the 10 or 9 litters whose mothers had been treated with 50 or 100 mg/kg, respectively, when compared with the 6 control litters. There was, however, a significant increase (p=0.01) in the liver weight and the liver-to-body weight ratio of the treated mice

TABLE 11-10

Toxic Effects of Pentachlorobenzene on Reproduction in Rats Dosed on Each of Gestation Days 6-15[a]

Parameter	Pentachlorobenzene Dose (mg/kg/day) 0	50	100	200
Number of rats pregnant at term	19	18	19	17
Live fetuses per litter	12.1	12.5	11.5	10.7
Fetal death (%)[b]	1.3	4.2	3.1	3.2
Fetal mean body weight (g)	4.8	4.9	4.8	4.4

[a] Source: Khera and Villeneuve, 1975

[b] Percent fetal death = (no. dead plus deciduomas) x 100/total no. of implants

TABLE 11-11

Skeletal and Soft-Tissue Abnormalities Observed in Rat Litters of Dams Treated with Pentachlorobenzene on Each of Gestation Days 6-15*

Parameter	Pentachlorobenzene Dose (mg/kg/day) 0	50	100	200
Skeletal Defects				
No. of fetuses examined	127	129	122	100
Extra ribs: unilateral	2	18	10	17
bilateral	2	10	11	46
Fused ribs	NA	NA	NA	2
Wavy ribs	5	2	NA	NA
Sternal defects	5	4	NA	31
Exencephaly	NA	NA	NA	NA
Soft-Tissue Anomalies				
No. of fetuses examined for visceral defects	67	69	67	52
Runts	1	2	NA	2
Cleft palate	NA	1	NA	NA
Cardiac defects	NA	NA	NA	NA
Other Defects	NA	NA	NA	2

*Source: Khera and Villeneuve, 1975

NA = No abnormality observed

TABLE 11-12

Fetal Wistar Rat Residues of Pentachlorobenzene[a,b]

Maternal Intubated Dose Level (mg/kg)	Whole Fetus[c] (ppm)	Whole Fetus[c] (Total μg)	Liver[d] (ppm)	Brain[d] (ppm)
50	2.44 ± 0.38	9.65 ± 1.3	4.37 ± 0.69	3.08 ± 0.55
100	5.27 ± 0.60	21.2 ± 2.1	10.4 ± 1.31	5.31 ± 0.60
200	16.9 ± 2.8	55.1 ± 6.7	40.4 ± 6.02	20.5 ± 2.64

[a] Source: Adapted from Villeneuve and Khera, 1975

[b] Pregnant rats were exposed to pentachlorobenzene during days 6-15 of gestation and the fetuses were removed and analyzed on day 22.

[c] Represents the mean of two fetuses from 15 litters ± s.e.m.

[d] Represents the mean of five fetuses each from a different litter ± s.e.m.

compared with the control mice. Pentachlorobenzene had no adverse effect on fetal development or survival. One fetus in the 50 mg/kg dose group displayed a cleft palate, but the occurrence was within the normal incidence for this strain of mice.

11.4. INTERACTIONS

Ariyoshi et al. (1975) and Goerz et al. (1978) demonstrated the ability of pentachlorobenzene to increase the activity of NADPH-cytochrome P-450 dependent enzyme systems in rats. Induction of the cytochrome P-450 monoxygenase-catalyzed metabolism could result in an increase or decrease in the toxicity of the compound. Therefore, exposure to pentachlorobenzene could result in the biotransformation and toxicity of drugs and other chemicals. However, no studies were available to support this.

11.5. SUMMARY

Pentachlorobenzene is absorbed from the gastrointestinal tract; studies indicated that 50-95% of an administered dose is absorbed within 4 days. One study that measured absorption through the skin suggested that pentachlorobenzene was poorly absorbed. No studies were available that measured absorption through the lungs.

Distribution is to many tissues, primarily the fat, liver and bone marrow. Transfer across placental membranes and excretion into the milk probably occur.

Metabolism is believed to be by oxidation to phenolic compounds, especially pentachlorophenol, that are excreted in the urine. Excretion appears to occur slowly; an estimated half-life in primates is 2-3 months.

No data were available on the effects of exposure to pentachlorobenzene in humans, and no chronic or carcinogenicity studies were available for review.

Oral LD_{50} values were determined for adult rats (1080-1125 mg/kg) and mice (1175-1370 mg/kg), and for weanling rats (940 mg/kg). No clinical signs of toxicity were observed in adult rats following dermal application of 2500 mg/kg pentachlorobenzene. Also, it was demonstrated that pentachlorobenzene caused an increase in the liver content of cytochrome P-450, microsomal drug metabolizing enzymes and microsomal proteins.

A subchronic feeding study indicated that the primary toxic effects are on the liver and kidneys, although slight changes in some hematologic parameters (e.g., decreased erythrocyte count, hemoglobin and hematocrit; increased leukocyte count) occurred in the high-dose groups. Histologic examination identified pathologic changes in the livers of the female rats fed 500 and 1000 ppm for 180 days and in the 1000 ppm male rats treated for 100 days. These data were sufficient to identify a subchronic LOAEL of 500 ppm (~27-63 mg/kg/day) and a NOEL of 250 ppm (~16-31 mg/kg/day).

No mutagenic activity was detected in five strains of Salmonella typhimurium when tested at five unspecified concentrations of pentachlorobenzene in the presence and absence of rat liver microsomes induced by Aroclor 1254. These results were reported in an abstract with few experimental details. Also, a negative result for pentachlorobenzene is not unexpected, because the Salmonella assay is generally insensitive to chlorinated compounds.

Studies also have shown that pentachlorobenzene is capable of causing reproductive and developmental effects. Female rats fed diets containing pentachlorobenzene during mating and gestation produced litters with reduced pup survival and body weights at weaning, and increased liver-to-body weight ratios. No adverse effects were observed in the offspring of the dams exposed to 125 ppm (6-16 mg/kg/day).

Single oral doses of pentachlorobenzene given daily to pregnant rats during gestation increased the incidence of fetal death at all doses tested, identifying a LOAEL of 50 mg/kg/day. Sternal defects and an increase in the incidence of extra ribs also were observed at doses of 200 mg/kg/day and 50, 100 and 200 mg/kg/day, respectively.

In a study of possible reproductive and teratogenic effects, doses of 50 and 100 mg/kg/day of pentachlorobenzene administered by gavage to pregnant mice had no adverse effect on fetal development or survival.

12. HEXACHLOROBENZENE

Hexachlorobenzene is not manufactured as a commercial product in the United States, but an estimated 2-5 million pounds were produced each year during the synthesis of several chlorinated chemicals (Mumma and Lawless, 1975). Hexachlorobenzene is also an ingredient in a fungicide of which ~200,000 pounds are imported each year (IARC, 1979). Hexachlorobenzene is resistant to biodegradation, accumulates in the biological environment and has been detected in ambient air, drinking and surface water, sediments, cropland and food (see Section 4.3.). Hexachlorobenzene residues also have been found in samples of human blood, fat and breast milk. The greatest degree of human exposure is most likely to occur in the workplace and near manufacturing and disposal sites, although the general population is likely to be exposed through inhalation of polluted air and the ingestion of contaminated food and water.

12.1. PHARMACOKINETICS

12.1.1. Absorption. Absorption of hexachlorobenzene from the gut has been studied in detail; however, no information has been found in the available literature on hexachlorobenzene absorption through the lungs or skin. Absorption of hexachlorobenzene from the intestinal tract appears to depend on the solvent vehicle used during test material administration. Thus, when hexachlorobenzene is administered in olive oil, ~80% of the dose is adsorbed; when it is administered in an aqueous solution, in 1% methyl cellulose, or in a crystalline form, relatively little (<20%) is absorbed. Intestinal absorption of hexachlorobenzene occurs primarily through lymphatic channels (Iatropoulos et al., 1975), with only a minor portion being absorbed into the portal circulation.

Ingebrigtsen et al. (1981) investigated the absorption of [^{14}C]hexachlorobenzene (10 mg in peanut oil) administered to male, bile-duct-cannulated Wistar rats by gastric catheter. Four days after dosing, 24.8% of the administered ^{14}C had been recovered in the feces, indicating that at least 75% of the administered hexachlorobenzene was absorbed.

Albro and Thomas (1974) studied the absorption of hexachlorobenzene in a squalane/cotton seed oil vehicle by male rats following administration of a single dose by stomach intubation. The results indicated that at doses of 12 and 30 mg/kg, ~82 and 72%, respectively, were absorbed within 96 hours.

Koss and Koransky (1975) compared the absorption rates of [^{14}C]hexachlorobenzene in female Wistar rats following oral administration of olive oil solutions or suspensions in 6% gum arabic in water (4, 20, 50.5, 60 and 180 mg/kg). Approximately 80% of the dose was absorbed from the olive oil solutions; however, only 6% was absorbed from the aqueous suspension.

Similarly, Zabik and Schemmel (1980) found that, when hexachlorobenzene (32 mg/kg/day) was administered in the diet, high-fat (45.3% w/w) diets resulted in greater accumulation of hexachlorobenzene in the tissues and less hexachlorobenzene excreted in the feces than did high-carbohydrate diets (67.7% w/w). The female rats received 32 mg/kg body weight hexachlorobenzene/day for 6, 12 or 18 days. Although this study did not include a control group receiving a balanced diet, the data suggest that high fat diets increase the absorption of hexachlorobenzene.

Sundlof et al. (1982) administered seven consecutive daily oral doses of 10 or 100 mg crystalline hexachlorobenzene/kg body weight to male laboratory beagles. The results from the 100 mg/kg group indicated that hexachlorobenzene can continue to be absorbed from the intestines for up to 1 week following the cessation of dosing.

Bleavins et al. (1982) fed female European ferrets (*Mustela putorius furo*) a single dose of 57.6 μg hexachlorobenzene (^{14}C-labeled) in 7.5 g of standard mink diet (22% fat) and calculated that 98.5% of the hexachlorobenzene dose was absorbed by the ferrets. They made this calculation based on predicted hexachlorobenzene excretion as extrapolated from this study, and owing to a food passage time in the female ferret of just over 3 hours.

12.1.2. Distribution. Following intestinal absorption, hexachlorobenzene, which is lipophilic, distributes to tissues that are rich in lipid content. The adipose tissue accumulates the greatest concentrations of hexachlorobenzene in all species studied, although bone marrow and skin, which contain large amounts of lipids, also accumulate hexachlorobenzene. The adrenal cortex accumulates hexachlorobenzene at concentrations approaching those of fat. Other tissues (e.g., kidneys, lungs, heart, spleen and blood) generally contain lower amounts of hexachlorobenzene. Intravenous injection of hexachlorobenzene results in a tissue distribution similar to that following oral administration. Hexachlorobenzene is transported via the placenta and is distributed in fetal tissue.

Mehendale et al. (1975) studied the disposition of ^{14}C-hexachlorobenzene by adult male rats following a single oral dose of 5 mg/kg. ^{14}C-Hexachlorobenzene was mixed with arachis oil and administered by stomach intubation at a dose of ~5 mg/kg. The animals were sacrificed 7 days later and the tissues and organs radioassayed. Forty-three percent of the total radioactivity administered was present in fat tissue 7 days after ^{14}C-hexachlorobenzene administration. In addition, muscle and skin tissues each contained ~9% of the radioactivity, whereas the other 12 tissues analyzed contained ~5% combined (Table 12-1).

TABLE 12-1

Storage and Excretion of ^{14}C-HCB Administered Orally in Arachis Oil in Rats[a]

Organ or Tissue	Percent of Total Radioactivity Administered
Fat[b]	42.81 ± 6.14
Muscle[c]	9.41 ± 1.17
Skin[d]	8.64 ± 1.21
Liver	3.01 ± 0.23
Small intestine	2.43 ± 0.47
Bone[e]	1.04 ± 0.09
Kidneys	0.76 ± 0.11
Large intestine	0.43 ± 0.08
Stomach	0.36 ± 0.04
Blood	0.24 ± 0.04
Lungs	0.24 ± 0.04
Testes	0.21 ± 0.04
Heart	0.18 ± 0.03
Brain	0.17 ± 0.03
Spleen	0.04 ± 0.002
Total in tissues	70.09 ± 5.48
Excretion	
Feces	16.02 ± 2.31[f]
Urine	0.85 ± 0.13[f]
Gut contents	2.48 ± 0.45
Total recovery	89.44 ± 10.57

[a] Source: Mehendale et al., 1975

[b] Based on 9% body weight as fat

[c] Based on 50% body weight as muscle

[d] Based on 16% body weight as skin

[e] Based on 10% body weight as bone

[f] Cumulative total for 7 days

Adult male rats were given 5 mg/kg of hexachlorobenzene.

HCB = Hexachlorobenzene

When ^{14}C-hexachlorobenzene was suspended in 1% methyl cellulose and a single oral dose containing 150 μg of hexachlorobenzene was administered to Sprague-Dawley rats, the absorption of ^{14}C-hexachlorobenzene by the walls of the stomach and duodenum 1 hour later was relatively low: ~1.0 and 0.6 ppm were found in the stomach and duodenum, respectively (Iatropoulos et al., 1975). Increased radioactivity was found in the jejunum and ileum as well as the lymph nodes and adipose tissues 3 hours after administration (Table 12-2). Although the radioactivity also increased in the liver and kidneys, this increase was relatively low compared to that found in the lymph nodes and adipose tissue. Moreover, the radioactivity in the liver and kidneys decreased within a 2-day period, whereas the radioactivity in the lymph nodes and fat remained relatively constant. These results indicate that the portal venous transport of hexachlorobenzene to the liver appears to be a minor pathway, whereas the major part of the ingested hexachlorobenzene is absorbed by the lymphatic system in the duodenum and jejuno-ileum and deposited in the fat, bypassing the systemic circulation and the excretory organs.

Knauf and Hobson (1979) investigated the tissue distribution of hexachlorobenzene in six female rhesus monkeys following the administration of daily doses of hexachlorobenzene [0 (one monkey), 8 (one monkey), 32 (one monkey), 64 (one monkey), or 128 (two monkeys) mg/kg/day] in 1% methyl cellulose for a period of 60 days. The highest concentrations of hexachlorobenzene were located in tissues with high lipid content. Tissue levels correlated more with body fat content than with dose, with the monkey that had the least adipose tissue producing the highest nonfat tissue and serum values (Table 12-3).

TABLE 12-2

Tissue Concentration (ppm) of ^{14}C-Hexachlorobenzene[a] and Its Metabolites in Sprague-Dawley Rats[b]

Tissue	Time (hours)									
	1		3		5		12		48	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Stomach	0.6	1.6	0.8	1.0	1.1	0.5	0.1	0.1	0.1	0.1
Duodenum	0.6	0.6	1.4	1.0	0.2	0.3	0.1	0.1	0.1	0.1
Jejuno-Ileum	0.1	0.2	0.6	0.8	1.0	0.3	0.3	0.3	0.2	0.1
Cecum	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.2	0.1	0.1
Colon	0.1	0.1	0.1	0.2	0.4	0.1	0.1	0.1	0.2	0.1
Liver	0.1	0.4	0.5	0.5	0.2	0.3	0.2	0.2	0.1	0.2
Mesenteric lymph node	0.1	0.6	0.4	1.3	2.0	1.0	1.5	1.0	1.9	2.1
Adipose tissue	0.1	0.2	1.7	1.2	2.3	1.5	1.3	1.1	2.6	2.7
Kidneys	0.1	0.2	0.4	0.3	0.5	0.2	0.2	0.1	0.2	0.1
Lungs	0.1	0.3	0.3	0.4	0.2	0.2	0.1	0.1	0.1	0.2

[a] 150 μg hexachlorobenzene was administered by stomach tube suspended in 1% methyl cellulose.

[b] Source: Iatropoulos et al., 1975

TABLE 12-3

Tissue Levels of HCB (ppm) in Adult Female Rhesus Monkeys[a,b]

Monkey No. Dose (mg/kg/day)	613[c] 128	618[d] 128	627[e] 64	817 32	1163 8	1826 0
Body fat	930	215	540	250	580	1.1
Bone marrow	460	175	1700	255	350	1.6
Adrenal cortex	150	30	325	90	50	0.1
Adrenal medulla	12	9	285	35	4	<0.1
Liver	20	50	365	40	30	<0.1
Kidney	18	19	258	11	3	<0.1
Brain	25	19	108	12	8	<0.1
Ovaries	6	23	133	3	1	<0.1
Muscle	4	21	24	7	2	<0.1
Serum	2.5	1.5	11.0	0.5	3.3	<0.1

[a] Source: Knauf and Hobson, 1979

[b] HCB was administered daily for 60 days in 1% methylcellulose

[c] Monkey was small and slight

[d] Monkey was obese

[e] Monkey had very little adipose tissue

HCB = Hexachlorobenzene

The highest levels of hexachlorobenzene residues were found in fat tissue (215-930 ppm) and bone marrow (175-1700 ppm), and selectively higher levels were found in the adrenal cortex (30-325 ppm) than in the adrenal medulla (4-285 ppm). Residues in semen, muscle, ovaries, brain, kidneys and liver were relatively much lower (0.5-258 ppm).

Engst et al. (1976) reported the administration by gavage of 8 mg/kg of hexachlorobenzene in 1 mℓ of sunflower oil to male Wistar rats for 19 days. The animals were then sacrificed, and the liver, kidneys, adrenals, heart, spleen and intestinal fat were analyzed for hexachlorobenzene residues. The following results were reported: fat tissue, 82 μg/g; muscle, 17 μg/g; liver, 125 μg total; kidneys total 21 μg each; spleen total 9 μg; heart total 1.5 μg; and adrenals total 0.5 μg each. High levels of hexachlorobenzene residues in fat tissues also have been reported for rats receiving 50.0 mg/kg (177 μmoles/kg) of hexachlorobenzene every second day for 10 weeks (Koss et al., 1980b).

Szymczynski and Waliszewski (1981) analyzed human semen and testicular and fat tissues, and identified several chlorinated pesticides that included hexachlorobenzene. The compound was not detected in testicular tissue, but was present in semen and fat tissues at concentrations of 0.001 and 0.128 μg/g, respectively. Similarly, hexachlorobenzene was one of several chlorinated compounds found in semen collected in 1979 from 132 college students (Dougherty et al., 1981).

Sundlof et al. (1982) studied the distribution of ^{14}C-hexachlorobenzene or unlabeled hexachlorobenzene in male beagles following a single intravenous dose of 1 mg/kg in olive oil. Two dogs each were sacrificed after 2, 4, 8, 16, 32 and 48 hours and after 12 weeks; hexachlorobenzene concentrations were determined in 16 tissues and organs as well as in the blood (Table 12-4). Two hours after dosing, the highest concentration

TABLE 12-4

HCB Concentrations in Tissues of Male Beagles Receiving Single Intravenous Doses of 1 mg/kg bw in Olive Oil*

Tissue	HCB Concentration (ppm) Time Interval After Dosing 2 hours	48 hours	12 weeks
Lungs	36.14	0.08	<0.01
Adrenals	2.82	0.38	0.06
Subcutaneous fat	1.14	3.38	0.37
Perirenal fat	1.00	3.24	0.46
Mesenteric fat	0.56	2.40	0.41
Spleen	0.54	0.01	<0.01
Liver	0.51	0.04	0.02
Thyroid	0.37	NR	0.02
Heart	0.28	0.04	0.01
Kidneys	0.18	0.02	0.01
Stomach	0.18	0.36	0.01
Pancreas	0.17	0.06	0.07
Brain	0.15	0.02	0.02
Duodenum	0.12	0.02	0.02
Colon	0.12	0.01	<0.01
Small intestine	0.07	0.02	0.01
Blood	0.07	0.03	0.01

*Source: Sundlof et al., 1982

NR = Not reported

HCB = Hexachlorobenzene

was found in the lungs (36.14 ppm). This was considered to be a property of the injection vehicle rather than a property of hexachlorobenzene per se. That is, it was believed that the olive oil vehicle formed microemboli in the blood which became trapped in the capillaries of the lung. Residue levels in the lungs then dropped (4.4 ppm), and a concurrent increase in hexachlorobenzene was detected in fat tissues (10.32 ppm in subcutaneous, perirenal and mesenteric fat) 4 hours postinjection. Residues in all tissues, organs and blood declined during the 48 hours postinjection except for fat tissue, which remained constant. Twelve weeks after dosing, tissue concentrations were very low in all tissues, including fat (>0.01-0.46 ppm), indicating significant excretion of the compound.

Yang et al. (1978) studied the distribution of hexachlorobenzene in male Sprague-Dawley rats and female rhesus monkeys following intravenous injection of ^{14}C-hexachlorobenzene in 1,2-propanediol:plasma (1:8). Rats received 0.1 mg of ^{14}C-hexachlorobenzene and then were replaced in metabolic cages for 48 hours before sacrifice. About 0.2 and 1.0% of the administered dose was excreted in the urine and feces, respectively. No radioactivity was exhaled from the animals. Over 20 tissues from the rats were analyzed and all were found to contain radioactivity. The highest levels were in fat (~3 μg/g of tissue). The adrenal glands also contained a relatively high level of radioactivity, whereas the other tissues contained much lower levels, generally in the range of 1/12 to 1/300 of those in fat tissue.

The tissue distribution of ^{14}C-hexachlorobenzene in rhesus monkeys was determined in individual animals 100 days, 6 months and 1 year after intravenous injection of ^{14}C-hexachlorobenzene at 0.38, 0.32 and 0.22 mg/kg, respectively. The results again indicated that the highest levels were present in fat (828-6069 ng/g) and bone marrow (373-1638 ng/g) among the 30 tissues analyzed in all three monkeys. The adrenal glands contained ~1/6 to 1/8 of the levels present in fat, whereas the other tissues contained radioactivity levels ranging between 1/10 to <1/800 of those in fat.

The transplacental transfer of hexachlorobenzene from pregnant mice, rats and rabbits has also been reported. Brandt et al. (1980) conducted a qualitative study on the distribution of ^{14}C-hexachlorobenzene and several of its sulfur-containing metabolites in pregnant mice. The mice were injected i.v. and sacrificed at intervals ranging between 20 minutes and 32 days after injection. The animals were frozen, sectioned and submitted to autoradiography. The autoradiograms showed a strong uptake of hexachlorobenzene in the adipose tissues. This hexachlorobenzene was found to persist in the adipose tissues for more than 1 month after the administration. Radioactive hexachlorobenzene was also found to penetrate the placenta, resulting in the blood and liver concentrations in the fetus equaling those of the dams.

Villeneuve and Hierlihy (1975) studied the placental transfer of hexachlorobenzene in Wistar rats and reported that hexachlorobenzene crosses the placenta and accumulates in the fetus in a dose-dependent manner. The females were dosed daily (5, 10, 20, 40 and 80 mg/kg) from gestation day 6-16 and then sacrificed on day 22. Only liver, brain and whole fetus residue levels were determined in this study. Fetal liver residues (1.8-35.8 μg/g) were much lower than those of the dams (9.3-86.0 μg/g). The fetal brain and whole fetus levels were 1.1-17.5 μg/g and 1.5-18.9 μg/g, respectively.

Villeneuve et al. (1974) also reported that the transplacental transport of hexachlorobenzene in New Zealand rabbits was dose-dependent. Rabbits were mated and then treated orally with hexachlorobenzene from days 1-27 with subtoxic doses of 0, 0.1, 1.0 or 10 mg/kg. On day 28 the dams were killed for fetal and maternal tissue analysis for hexachlorobenzene. In dams, the hexachlorobenzene residue concentrations were highest in fat, followed by the liver, heart, kidneys, brain, lung, spleen and plasma. Hexachlorobenzene residues were higher in the fetal liver than in the maternal liver.

Courtney et al. (1976) reported on the distribution of hexachlorobenzene (assayed 90.4% hexachlorobenzene and 9.6% pentachlorobenzene) administered on days 7-11 of gestation at a dose of 50 mg/kg/day in a corn oil acetone mix to five pregnant and two non-pregnant CD-1 mice. They found there were no remarkable differences in the hexachlorobenzene tissue levels between the pregnant and non-pregnant animals sampled at day 12 of pregnancy. The levels of pentachlorobenzene in sampled tissues were low as compared to the very high hexachlorobenzene levels detected in the thymus, skin, fat and urinary bladder. No detectable levels of hexachlorobenzene or pentachlorobenzene were found in the control mice.

Courtney et al. (1979) studied the tissue distribution of hexachlorobenzene in the maternal and fetal tissues of CD rats and CD-1 mice and reported that placentas and fetuses of both species demonstrated a dose-dependent relationship for hexachlorobenzene residues, with levels in the fetuses being higher than those in their corresponding placentas. The dams were treated with single or multiple oral doses (10, 50 or 100 mg/kg in corn oil) at different periods during gestation. The hexachlorobenzene concentrations in mice and rat fetuses at mid-gestation were very similar. In mice, multiple low doses of hexachlorobenzene resulted in higher concentrations of hexachlorobenzene in maternal and fetal tissues than single doses of equivalent total doses. In another study, Courtney and Andrews (1979) reported that in mice the fetus could be exposed to hexachlorobenzene from maternal body burdens, established before fetal implantation, and was not limited to maternal exposure during the postimplantation gestation.

Bleavins et al. (1982) studied the tissue distribution and transfer of a single dose of hexachlorobenzene given to female European ferrets (Mustela putorius furo). They gave a single 57.6 μg hexachlorobenzene (^{14}C-labeled) dose to each of three bred and five non-bred ferrets, in 7.5 g of standard mink diet (22.2% fat). The dosed ferrets and offspring were observed for 5 weeks after the kits were born, at which time they were killed and tissue ^{14}C-hexachlorobenzene levels were determined (Table 12-5). One ferret kit per litter was also collected at birth and at weeks 1, 2, 3 and 4 for whole body residue determinations (Table 12-6). These results indicate that nursing mothers can significantly reduce their body burdens of hexachlorobenzene, when compared to unbred female counterparts, by transferring a large amount of the hexachlorobenzene to their offspring. The mothers' milk contaminated with hexachlorobenzene seems to be a large contributor to the kits' body burdens with a reported milk to placental exposure ratio of 31:1. The distribution of hexachlorobenzene in ferrets follows similar trends, as observed in the other mammals, of the highest hexachlorobenzene levels being found in the lipid rich tissues.

The transfer of hexachlorobenzene to nursing infant rhesus monkeys from lactating mothers receiving 64 mg/kg/day for 60 days was reported by Bailey et al. (1980). Milk concentrations were on the average 17-fold higher than

TABLE 12-5

Mean (±SE) Hexachlorobenzene Radioactivity (dpm/g) of Selected European Ferret Tissues[a,b]

Tissues	Group I (n=3)	Group II (n=5)	Kits[c] (n=3)
Blood	49 ± 34.6[d]	166 ± 26.8	--
Subcutaneous fat	4472 ± 780.5[e]	19,525 ± 1503.9	11,678 ± 712.4[f]
Visceral fat	4429 ± 867.6[e]	19,704 ± 1666.0	--
Muscle	53 ± 14.4[d]	384 ± 64.0	561 ± 204.8
Heart	34 ± 9.2[d]	310 ± 56.8	--
Kidney	105 ± 31.1[e]	611 ± 80.4	209 ± 37.2
Spleen	13 ± 7.5[e]	180 ± 24.8	--
Liver	248 ± 68.9[e]	1,445 ± 145.2	1,420 ± 185.6[g]
Lung	1 ± 0.3[e]	241 ± 18.4	--
Brain	61 ± 30.0[e]	395 ± 48.5	130 ± 29.4

[a] Source: Bleavins et al., 1982

[b] at 62 days postdosing from adult bred (group I) and unbred (group II) female ferrets exposed to a single 57.6 μg dose of ^{14}C-labeled hexachlorobenzene and from offspring born to the bred females.

[c] Kit tissues, from 5-week-old offspring, were contrasted only with maternal (group I) tissues.

[d] Significantly different ($p<0.05$) from group II tissue of the same type.

[e] Significantly different ($p<0.01$) from group II tissue of the same type.

[f] Significantly different from maternal tissue (group I) at $p<0.01$.

[g] Significantly different from maternal tissue (group I) at $p<0.05$.

HCB = Hexachlorobenzene

TABLE 12-6

Mean (±SE) HCB Radioactivity (dpm x 10^3) of European Ferret Kits[a,b]

Measure	Number	Weeks Postpartum					
		0	1	2	3	4	5
Per gram of kit	3	3.0 ± 0.19	2.7 ± 0.57	4.3 ± 0.67	3.9 ± 0.73	3.5 ± 0.50	2.7 ± 0.14
Per whole kit	3	25.1 ± 1.43	76.7 ± 14.35	311.4 ± 63.39	492.5 ± 92.22	672.8 ± 117.63	805.7 ± 54.25
Increase over previous week		--	51.6	234.7	181.1	180.3	132.8
Milk (per mℓ)	3	--	--	6.1 ± 0.66	2.9 ± 0.45	1.8 ± 0.17	0.8 ± 0.20

[a] Source: Bleavins et al., 1982

[b] Born to female ferrets exposed to a single dose of ^{14}C-labeled hexachlorobenzene and the milk produced by those dams

HCB = Hexachlorobenzene

maternal serum levels, whereas infant serum levels were about 2- to 5-fold higher than serum levels of their mothers. Similarly, the infants had higher tissue residues than their mothers and hexachlorobenzene was concentrated in the infant fat, bone marrow, adrenals and lymph nodes.

Hexachlorobenzene residues also have been reported in human fat in the United Kingdom (Abbott et al., 1981, Japan (Curley et al., 1973), and Australia (Brady and Siyali, 1972) and in human milk collected in Sweden (Westoo and Noren, 1978; Hofvander et al., 1981), Canada (Mes and Davies, 1979), Norway (Bakken and Seip, 1976; Skaare, 1981), and Hawaii (Takahashi et al., 1981).

12.1.3. **Metabolism.** The metabolism of hexachlorobenzene has been studied in male and female rats following oral administration, rhesus monkeys and beagles following i.v. injection, and rabbits following i.p. injection (Renner, 1981). Hexachlorobenzene is metabolized slowly into other chlorinated benzenes, chlorinated phenols and other minor metabolites and forms glucuronide and glutathione conjugates. Tissues were found to contain mainly unchanged hexachlorobenzene together with small amounts of metabolites. Similarly, only small amounts of hexachlorobenzene metabolites were detected in feces, whereas most of the metabolites were excreted in the urine together with small amounts of unchanged hexachlorobenzene.

Mehendale et al. (1975) studied the metabolism of hexachlorobenzene in male Sprague-Dawley rats 7 days after oral administration of a single 5 mg/kg dose. The fat, liver, intestines, kidneys, lungs and brain were found to contain hexachlorobenzene primarily, along with trace amounts of other chlorinated benzenes. Analysis of these chlorinated benzenes suggested the presence of pentachlorophenol, 2,4,5-trichlorophenol, pentachlorobenzene and the tetrachlorobenzenes. Extraction and analysis of fecal radioactivity, which accounted for 16% of the dose, did not reveal the presence of metabolites. Although urine contained only 0.85% of the administered radioactivity, it provided the only evidence of hexachlorobenzene metabolite excretion. Several unidentified metabolites were evident following thin-layer chromatography (TLC) separation of urine, in addition to 2,4,5-trichlorophenol, pentachlorophenol and one spot was reported to contain a mixture of chlorinated benzenes.

In vitro metabolism studies with homogenates of the liver, lungs, kidneys and small intestines produced trace amounts of chlorobenzene metabolites when incubated with [^{14}C]-hexachlorobenzene in the presence or absence of added cofactors. Liver microsomal preparations produced amounts of one or more chlorophenols when fortified with NADPH; in the presence of UDPGA, pentachlorophenol was reported to form the glucuronide conjugate. Fortification of kidney homogenates with glutathione resulted in the appearance of unextractable radioactivity in the aqueous phases, indicating that glutathione conjugates of polar hexachlorobenzene metabolites might also be formed (Mehendale et al., 1975).

The metabolism of hexachlorobenzene in male and female Sprague-Dawley rats each receiving nine doses of 85.6 mg/kg hexachlorobenzene (99.7% pure) in arachis oil over a period of 1 month was reported by Richter et al. (1981). The animals were sacrificed 3, 24 and 52 days after the last dose, and various tissues were analyzed for hexachlorobenzene and its metabolites by CDE/GLC and GLC/MS. In addition to hexachlorobenzene, the following metabolites were also detected: pentachlorobenzene (PCB), pentachlorophenol (PCP), pentachlorothiophenol (PCTP) and 2,3,4,6- and 2,3,5,6-tetrachlorophenol (TCP). The results reported for the liver and kidneys for day 3 indicated that the livers of the females contained significantly more PCTP, a derivative of a glutathione conjugate, than those of the males (Table 12-7). However, it is not known whether that increase is due to a higher rate of PCTP production or to a lower rate of elimination.

Rizzardini and Smith (1982) investigated the sex differences in hexachlorobenzene metabolism in young F344/N rats who had been intubated every other day for 103 days with 14 mg/kg hexachlorobenzene (analytical grade) dissolved in arachis oil. Three hexachlorobenzene metabolites were analyzed for: pentachlorobenzene, pentachlorothiophenol and 2,3,5,6-tetrachlorobenzene-1,4-diol, and all three were found to be produced in larger concentrations in the female rats during the first 10 weeks of hexachlorobenzene treatment. The greater quantities of hexachlorobenzene metabolites being formed in female rats was believed due to their body estrogen levels.

Engst et al. (1976) detected several urinary metabolites in male Wistar rats receiving by gavage 8 mg/kg of hexachlorobenzene daily dissolved in sunflower oil for 19 days. The results of this study were presented qualitatively, and the authors reported that the major metabolic route for hexachlorobenzene was to pentachlorophenol. In addition, the feces contained mainly unchanged hexachlorobenzene together with traces of pentachlorobenzene.

Koss et al. (1976) investigated the metabolism of hexachlorobenzene in female Wistar rats given 2-3 i.p. doses of [^{14}C]hexachlorobenzene (260 or 390 mg/kg total dose). At the end of 4 weeks, 7% of the administered radioactivity was excreted in the urine, with >90% of this amount contained in the major metabolites (pentachlorophenol, tetrachlorohydroquinone, and pentachlorothiophenol). An isomer of tetrachlorothiophenol was detected as a minor urinary metabolite. Twenty-seven percent of the administered radioactivity was excreted in the feces, of which 70% was identified as unchanged

TABLE 12-7

Concentrations of HCB and its Metabolites (mg/kg) in the Liver and Kidneys of Male and Female Rats[a,b]

Tissue/Sex	HCB	PCB	PCP	PCTP	TCP
Liver					
Males	192	0.05	3.16	0.23	0.02
Females	147[c]	0.03[c]	2.12[c]	0.36[c]	0.04[c]
Kidneys					
Males	127	0.05	5.79	0.24	0.09
Females	111	0.01	3.69	0.10	0.08

[a] Source: Richter et al., 1981

[b] Determined 3 days after the last of nine oral doses of 85.6 mg/kg HCB given within 1 month in arachis oil

[c] Statistically significant from males ($p<0.05$)

HCB = Hexachlorobenzene; PCB = pentachlorobenzene; PCP = pentachlorophenol; PCTP = pentachlorothiophenol; TCP = 2,3,5,6-tetrachlorophenol hexachlorobenzene. Only pentachlorophenol and pentachlorothiophenol were identified as fecal metabolites of hexachlorobenzene. In the tissues of the animals, only pentachlorophenol was detected in measurable quantities, accounting for 10% of the radioactivity in blood and <0.1% in body fat. Total radioactivity contained in the metabolites detected in the animal bodies and excreted at the end of the 4 weeks accounted for 16% of the administered radioactivity.

In follow-up studies, Koss et al. (1978a) compared the formation of hexachlorobenzene metabolites in rats, mice, guinea pigs, Japanese quail, laying hens and rainbow trout. The only metabolites detected were pentachlorophenol, tetrachlorohydroquinone and pentachlorothiophenol; however, the species tested differed greatly in their ability to metabolize hexachlorobenzene (Table 12-8).

Gas-liquid chromatography of urine, bile and fecal extracts from male beagle dogs receiving a single i.v. injection of ^{14}C-hexachlorobenzene at 1 mg/kg revealed that 96% of the fecal radioactivity occurred as the parent compound. Hexachlorobenzene accounted for 4% of the biliary radioactivity, but no parent compound was detected in urine (Sundlof et al., 1982).

Kohli et al. (1976) studied the metabolism of several chlorinated benzenes, including hexachlorobenzene, in rabbits following i.p. injection. The urine was collected for 10 days after injection and analyzed for metabolites following extraction and gas-liquid chromatography, but no hexachlorobenzene metabolites were found in the urine.

12.1.4. Excretion. The excretion of hexachlorobenzene from treated animals is slow and occurs mainly through the feces, with relatively little being excreted in the urine. It is characterized by an initial rapid phase followed by a very slow phase. This slow phase of excretion can be enhanced

TABLE 12-8

Hexachlorobenzene and Its Major Metabolites in the Excreta of Different Animal Species[a]

Species[b]	Total Dose (mMol/kg)	Total Amount of Substances			
		HCB	PCP	TCH	PCTP
Rat	0.92	6.1[c]	2.0	0.4	1.8
Mouse	0.92	2.6	0.3	0.1	ND
Guinea pig	0.92	1.8	0.9	0.2	0.5
Japanese quail	2.76	7.5	trace	trace	3.2
Laying hen	0.92	0.6	0.1	0.07	0.04
Rainbow trout	2.76	1.8	0.4	ND	ND

[a] Source: Koss et al., 1978a

[b] 2-3 animals were used per each species investigated

[c] Figures are given in μMol/kg bw/day

ND = Not detected. The lower detection limit of the metabolites was determined to be 0.03 nMol/mℓ urine or g feces.

HCB = Hexachlorobenzene; PCP = pentachlorophenol; TCH = tetrachlorohydroquinone; PCTP = pentachlorothiophenol by the administration of mineral oil, paraffin and n-hexadecane. Both biliary and intestinal excretion contribute to fecal excretion. A three-compartment mammalian model has been reported for the behavior of hexachlorobenzene in beagles and rhesus monkeys following i.v. injection of a single dose. Radioactivity was not detected in exhaled air following i.p. injection of ^{14}C-hexachlorobenzene.

Studies conducted by Mehendale et al. (1975) with rats receiving a single oral dose indicated that only 16.0 and 0.85% were excreted in the feces and urine, respectively, 7 days after treatment (see Table 12-1). Ingebrigtsen et al. (1981) reported that 4 days after intragastric administration of ^{14}C-hexachlorobenzene, a total of 24.8 and 2.1% of the administered radioactivity were recovered in the feces and urine, respectively. In addition, an average of 3.6% of the dose was recovered in the bile of bile-duct-cannulated rats within 48 hours after dosing. Of the radioactivity excreted in the bile, only 2% was unchanged hexachlorobenzene, 1.8% was pentachlorobenzene, 24% was pentachlorophenol and ~72% was unidentified metabolites.

Rozman et al. (1977) studied the excretion of hexachlorobenzene in female rhesus monkeys receiving 110 μg ^{14}C-hexachlorobenzene/day/monkey via diet for 15 months. The excretion and storage patterns showed a very slow approach to a saturation level, indicating a high tendency for hexachlorobenzene accumulation in rhesus monkeys. A total of 5.8 and 3.6% of the administered dose was excreted in the urine of male and female monkeys, respectively, after 15 months, of which 50% was pentachlorophenol, 25%

pentachlorobenzene and the remaining 25% consisting of unidentified metabolites with varying amounts of hexachlorobenzene. A total of 47.9 and 27.5% of the dose was present in the feces of male and female monkeys, respectively, of which 99% was hexachlorobenzene.

Koszo et al. (1978) administered hexachlorobenzene (0.2% in the diet) to young male and female Wistar rats for as long as 200 days and measured the accumulation of hexachlorobenzene in the liver and fatty tissue and the excretion of hexachlorobenzene and pentachlorophenol in the urine and feces. The concentration of hexachlorobenzene in the liver and fat increased steadily throughout the treatment period. Pentachlorophenol appeared in both the urine and feces in increasing amounts throughout the treatment period, with the excretion of other apolar and polar products being markedly enhanced after 5-6 weeks.

Rizzardini and Smith (1982) investigated the sex differences in hexachlorobenzene metabolism and excretion of hexachlorobenzene metabolites in young F344/N rats. These rats were intubated with 14 mg/kg analytical grade hexachlorobenzene dissolved in arachis oil every other day for 103 days and were analyzed for the three main hexachlorobenzene metabolites, pentachlorophenol, pentachlorothiophenol and 2,3,5,6-tetrachlorobenzene-1,4-diol, in urine and feces. Results indicated that the combined urinary excretion of metabolites was greater in the female rats, especially during the first 10 weeks, with pentachlorothiophenol being particularly high in the females. No wide variations between the sexes were seen in the analyzed feces hexachlorobenzene metabolites after 103 days of treatment. Combined urine and feces excretion of metabolites at the end of the study were found not to be significantly different between males (2291±116 nmole/ 24 hours/kg) and females (2425±182 nmole/24 hours/kg). It was stated, though, that the total excretion of pentachlorothiophenol was always significantly higher in the female rats.

Koss and Koransky (1975) studied the metabolism of hexachlorobenzene in rats when the compound was orally administered in an aqueous suspension or in olive oil. The animals received different amounts of ^{14}C-hexachlorobenzene in a single dose, and the feces and urine were collected at varying time intervals and radioassayed. When administered in water, hexachlorobenzene was not readily absorbed; 76-97% of the dose was excreted in the feces, and <0.1-0.4% was excreted in the urine 1 day after administration. When administered in oil, only 45-46% of the dose was excreted in the feces and 2.1-3.8% was excreted in the urine after 14 days of treatment. Rats receiving 4 mg/kg of ^{14}C-hexachlorobenzene administered i.p. excreted a total of 5 and 34% of the dose in the urine and feces, respectively, within 14 days. About 4 and 80% of the excreted radioactivity in the urine and feces, respectively, was unchanged hexachlorobenzene. Animals injected i.p. with 50.5 mg/kg [^{14}C]hexachlorobenzene released no radioactivity in exhaled air (Koss and Koransky, 1975).

Rozman et al. (1981) reported that administration of mineral oil or n-hexadecane to female Sprague-Dawley rats or male or female rhesus monkeys who were pretreated with ^{14}C-hexachlorobenzene enhanced the fecal elimination of ^{14}C-hexachlorobenzene. All animals were administered ^{14}C-hexachlorobenzene (100 mg/kg) orally as a single dose except for one monkey that received three consecutive daily doses and two monkeys that received ^{14}C-hexachlorobenzene (0.11 mg/kg) in sugar pellets daily for 750 consecutive days. Aliphatic hydrocarbons were administered to the treated animals 11-40 days after hexachlorobenzene treatment. When mineral oil was added to the diet of the rhesus monkeys, fecal excretion was enhanced 6- to 9-fold. Similarly, dietary administration of hexadecane resulted in the same increase in fecal excretion of hexachlorobenzene in both the rhesus monkeys and rats. Residue analyses indicated an enhanced depletion of hexachlorobenzene from blood and of stored hexachlorobenzene from adipose tissue. Enhanced fecal excretion of hexachlorobenzene as a result of dietary administration of aliphatic hydrocarbons was primarily due to increased hexachlorobenzene elimination in the large intestine.

Richter and Schafer (1981) studied the intestinal excretion of hexachlorobenzene in male Sprague-Dawley rats using the pendular perfusion method. The animals were injected i.p. with hexachlorobenzene at 100 mg/kg and, 1 and 4 weeks after treatment, various parts of the intestines were perfused with paraffin or squalane for 5 hours. The largest amount of hexachlorobenzene excreted was into the jejunum followed by the ileum and colon. The ratios of total hexachlorobenzene excreted during paraffin treatment were: jejunum/ileum = 1.26 and jejunum/colon = 2.43. The authors concluded that these results indicate the importance of intestinal excretion in the elimination of hexachlorobenzene, and that paraffin treatment can be one of the measures by which a long-term stimulation of hexachlorobenzene excretion can be achieved.

Beagle dogs receiving a single i.v. dose of 1 mg/kg excreted 44 and <6% of the dose in the feces and urine, respectively, during a 12-week period (Sundlof et al., 1982). Both biliary and intestinal excretion contributed to fecal excretion; however, the data indicated that biliary excretion was the major contributor to fecal excretion. A computer-assisted pharmacokinetic analysis of blood, urine and fecal radioactivity levels during a

12-week period suggested a three-compartment model for the behavior of hexachlorobenzene in beagles. The biological half-life values were calculated for the three dogs used and ranged from 6 weeks to 3 years.

Yang et al. (1978) reported that the elimination rate of hexachlorobenzene from male Sprague-Dawley rats and rhesus monkeys injected i.v. with hexachlorobenzene was slow because hexachlorobenzene is stored in the fat tissue. The major route of radiolabel excretion in treated monkeys was via the feces. About 17.1, 8.8 and 28.2% of the dose was excreted in the feces after 100 days, 6 months and 1 year, respectively, after treatment of individual monkeys, with ~90% of the radioactivity determined to be unchanged ^{14}C-hexachlorobenzene. The cumulative urinary excretion of hexachlorobenzene metabolites was determined to be 1.6% of the administered dose after 1 year. An open system, three-compartment mammillary model was found to fit the data for plasma, fecal and metabolized hexachlorobenzene in the rhesus monkey.

Koss et al. (1983) administered 100 mg/kg hexachlorobenzene every other day, via stomach tube, to female Wistar rats for a period of 6 weeks and then observed the rats for an additional 18 months. At cessation of hexachlorobenzene treatment they tried to assess the biological half-life of hexachlorobenzene and determined a value of 8 days for the start of the elimination phase, a value of 10 weeks when assessed 3 months later, and finally a value of 1.5 years after 12 months. The authors then concluded that it is not possible to establish a valid biological half-life for the total elimination phase of hexachlorobenzene in rats.

Bleavins et al. (1982) studied the excretion and transfer of hexachlorobenzene given to female European ferrets (Mustela putorius furo). Three bred and five non-bred female ferrets were each given a single dose of 57.6

μg ^{14}C-hexachlorobenzene in 7.5 g of standard mink diet (22.2% fat). The investigators indicated that there were no significant differences in the excretion of hexachlorobenzene metabolites, between bred and non-bred groups, in urine for the entire 8-week study period or in feces during the beginning of the study. The observed fecal excretion during the middle weeks to the end of the study showed a leveling of the cumulative fecal excretion in the bred females and a continued increase in fecal excretion in the non-bred female ferrets, although it was stated that this difference was not statistically significant. Excretion of hexachlorobenzene or metabolites in the milk was found to be an important route of excretion for lactating females, ~20.3% of the initial dose was eliminated by the fifth week of lactation, and found to be a very important route of exposure to nursing offspring. The importance of placental transfer and milk excretion is further presented by observing the time required for 50% of the initial hexachlorobenzene dose to be excreted. The bred females required 32 days to excrete 50% while 41 days was required for the unbred females.

12.1.5. **Summary**. The pharmacokinetics of hexachlorobenzene in a number of mammalian species have been studied in detail following oral administration and, to a lesser extent, following i.v. or i.p. injection. No information was present in the available literature on hexachlorobenzene metabolism following inhalation or topical application. Absorption of hexachlorobenzene from the intestinal tract appears to depend on the solvent vehicle used during test material administration. Thus, when hexachlorobenzene is administered in olive oil, ~80% of the dose is absorbed; when it is administered in an aqueous solution, in 1% methyl cellulose or in a crystalline form, relatively little (<20%) is absorbed. Intestinal absorption of hexachlorobenzene occurs primarily through lymphatic channels, with only a minor portion being absorbed into the portal circulation.

Following absorption, hexachlorobenzene distributes to tissues that have a high lipid content. The adipose tissue accumulates the greatest concentrations of hexachlorobenzene in all species studied, although bone marrow and skin, which contain large amounts of lipids, also accumulate hexachlorobenzene. The adrenal cortex accumulates hexachlorobenzene at concentrations approaching those of fat. Other tissues (e.g., kidneys, lungs, heart, spleen and blood) generally contain much lower amounts of hexachlorobenzene. Intravenous injection of hexachlorobenzene results in a tissue distribution similar to that seen following oral administration. Hexachlorobenzene is transported via the placenta and is distributed in fetal tissue in rabbits, rats, mice, minks and ferrets.

Hexachlorobenzene is metabolized slowly into other chlorinated benzenes, chlorinated phenols and other minor metabolites and forms glucuronide and glutathione conjugates. Tissues were found to contain mainly unchanged hexachlorobenzene together with small amounts of metabolites. Similarly, only small amounts of hexachlorobenzene metabolites were detected in feces, whereas most of the metabolites were excreted in the urine together with small amounts of unchanged hexachlorobenzene. There are indications that females produce and excrete more hexachlorobenzene metabolites than do males.

The excretion of hexachlorobenzene from treated animals is slow and occurs mainly through the feces with relatively little being excreted in the urine. It is characterized by an initial rapid phase followed by a very slow phase. This slow phase of excretion can be enhanced by the administration of mineral oil, paraffin or n-hexadecane. Both biliary and intestinal excretion contribute to fecal excretion. A three-compartment mammillary model has been reported for the behavior of hexachlorobenzene in beagles and rhesus monkeys following i.v. injection of a single dose. Radioactivity was not detected in exhaled air following i.p. injection of ^{14}C-hexachlorobenzene. Hexachlorobenzene has been detected in the milk of nursing mammals (see Sections 12.1.2. and 12.2.).

12.2. EFFECTS ON HUMANS

The effects of hexachlorobenzene on humans as a result of accidental or occupational exposure have been reviewed by Courtney (1979) and Currier et al. (1980). A few reports of data collected on occupationally exposed workers have been reported with studies conducted in Turkey and in the United States (i.e., Louisiana) on the general population following accidental exposure to hexachlorobenzene. The exposure of humans to toxicologically significant levels of hexachlorobenzene in Turkey from 1955-1959 by ingestion of contaminated grain, as reported by Cam (1959, 1960), Cam and Nigogosyan (1963) and Peters (1966), caused an epidemic of hexachlorobenzene-induced porphyria cutanea tarda (PCT), also known as porphyria turcica.

12.2.1. Epidemiologic Studies. Burns et al. (1974) found 0-310 ppb hexachlorobenzene in blood samples from 20 vegetable spraymen. There were no signs of PCT, and no correlations were observed between hexachlorobenzene levels and urinary porphyrin excretion, serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvic transaminase or lactate dehydrogenase. Increased levels of urinary porphyrins were detected in 1 of 54 men occupationally exposed to hexachlorobenzene (Morley et al., 1973).

A medical survey was conducted by Dow Chemical Company (Currier et al., 1980) on 50 employees working at a chlorinated solvents plant in Louisiana, to determine blood hexachlorobenzene levels and signs suggestive of PCT or other adverse effects, as well as any changes in hematologic, clinical chemistry and urinalysis parameters. The results from this study are of limited value because the various parameters studied during the 4-year period were analyzed by several laboratories using various methods and on different individuals. There was potential exposure to other substances also. During various times of the study, the time-weighted-average airborne concentrations of hexachlorobenzene ranged from <1-13 ppb, and wipe samples in the control, laboratory and clerical work areas ranged from 0.03-1.24 $\mu g/100\ cm^2$.

The laboratory analyses and physical examinations performed on the 1977 study group and on a control group from a polyethylene plant did not reveal any signs indicative of PCT. Levels of hexachlorobenzene, urinary porphyrin and coproporphyrin and the average years of exposure are listed in Table 12-9. A statistically significant ($p<0.05$) correlation was found between hexachlorobenzene levels in blood and the number of years worked in the plant. For the other studied parameters no statistically significant differences were noted between the 44 chlorinated solvents workers and the 44 control workers for 1977, except for higher protein levels and lower hematocrit values in the former workers which were not considered to be biologically significant. In addition, significantly lower levels of urinary coproporphyrins and albumin were detected in white male workers with hexachlorobenzene blood levels ≥200 ppb than in those with hexachlorobenzene levels <200 ppb.

Burns and Miller (1975) studied plasma hexachlorobenzene residues of 86 residents living and/or working in an area exposed to the production, transportation and disposal of "hex" wastes (hexachlorobenzene and other chlorinated hydrocarbons) in Louisiana. Plasma hexachlorobenzene levels were measured and correlated with demographic characteristics, occupational hazards,

TABLE 12-9

Results of Blood and Urine Analysis in Men Employed in a Chlorinated Solvents Plant, 1974-1977[a]

Parameter	Study Group				Comparison Group
	1974 (n=50)	1975 (n=49)	1976 (n=49)	1977 (n=44)	1977 (n=44)
Blood HCB (µg/ℓ)	310.7 ± 287.7[b]	311.5 ± 242.9[b]	159.9 ± 142.7[c]	170.3 ± 111.8[c]	0.1 ± 0.6
Urinary uroporphyrins (µg/ℓ)	22.4 ± 21.1	20.9 ± 11.0	37.4 ± 14.4	26.2 ± 14.3	NR
Urinary coproporphyrins (µg/ℓ)	77.4 ± 40.5	67.2 ± 36.1	100.6 ± 40.8	95.2 ± 48.9	NR
Age (years)	30.1 ± 6.3	31.1 ± 6.6	30.8 ± 6.7	31.7 ± 7.1	31.3 ± 6.8
Plant-years	5.5 ± 3.9	6.3 ± 4.0	5.9 ± 4.5	6.6 ± 4.8	6.6 ± 4.4

[a] Source: Currier et al., 1980; 1974-1975 results conducted by Bioscience Laboratories; 1976-1977 results conducted by Pathology Laboratories (± Standard Deviation)

[b] In plasma

[c] In blood

N = Sample size

NR = Not reported

HCB = Hexachlorobenzene

food sample analyses and house dust analyses. Average plasma levels of hexachlorobenzene ranged from 2.4-3.6 ppb in exposed subjects as compared with 0.5 ppb in controls ($p<0.001$; Table 12-10).

Higher levels of hexachlorobenzene residues, which were statistically significant ($p<0.05$), were found in the male subjects (4.71 ppb) than in the female subjects (2.79 ppb). These were not associated with race or exposure to hexachlorobenzene through the consumption of homegrown vegetables and animals. About 68% of the house dust samples contained an average hexachlorobenzene concentration of 380 ppb as compared with 20 ppb in control samples. When the hexachlorobenzene levels in dust were compared with the mean plasma hexachlorobenzene residues for the same household, a significant correlation was obtained ($p<0.025$). In addition, blood samples from 11 workers employed for an average of 4.8 years (10 months to 15 years) at the chemical plant contained an average of 78.6 (14-233) ppb hexachlorobenzene.

12.2.2. Accidental Ingestion in Turkey. The hexachlorobenzene-induced PCT epidemic in Turkey, a result of exposure during 1955-1959 in individuals who used contaminated seed wheat for food, has been reviewed by Courtney (1979). Cam and Nigogosyan (1963) estimated that 0.05-0.2 g of hexachlorobenzene was consumed per day. The method of estimation was not described. PCT is a disease of disturbed porphyrin metabolism manifested by cutaneous lesions and is commonly followed by hypertrichosis (hairiness) and hyperpigmentation. The induction of porphyria by hexachlorobenzene has been reviewed (DeMatteis, 1967; Granick, 1965; Tschudy and Bonkowsky, 1972; Courtney, 1979). Porphyrias are metabolic disorders of porphyrin metabolism that are characterized by increased excretion of porphyrins and their precursors. Normally, δ-aminolevulinic acid synthetase is the rate-limiting step in porphyrin synthesis and heme acts as an end-product inhibitor or an end-

TABLE 12-10

HCB Plasma Levels in Exposed Individuals and Controls[a]

Parameter	Exposed[b]	Controls[b]
Number of subjects	86	43
Age (years)	39.8 ± 19.1	32.3 ± 18.6
Black/white ratio	1.0	2.3
HCB plasma residues (ppb)	2.4 ± 2.3[c]	0.5
Range (ppb)	0-23	0-1.8
Percent positive	99	95
Percent >1 ppb	99	5

[a] Source: Burns and Miller, 1975

[b] Values are mean ± 1 SD

[c] Level for random sample only, N=63 (3.6 ± 4.3 for random and biased samples, N=83)

HCB = Hexaclorobenzene

end-product repressor of δ-aminolevulinic acid synthetase. In hexachlorobenzene-induced porphyria, δ-aminolevulinic acid synthetase is induced but heme does not suppress or inhibit the enzyme. The activity of uroporphyrinogen decarboxylase is decreased, consequently, porphyrin and its precursors (e.g., uroporphyrinogen, coproporphyrinogen and occasionally series I porphyrins) are excreted mainly in the urine but also in the feces. Increased levels of porphyrins also can be measured in the liver, skin, intestinal tract and other tissues (Courtney, 1979). PCT appeared to occur more frequently in children 4-16 years of age, whereas the number of adults and children under 5 years of age reporting PCT was much lower (10-24% of cases were individuals over 15 years of age and <5% were children below the age of 4). A distinct disease described as "pink sore" was observed in children under 1 year of age and achieved an epidemic scale. The clinical symptoms were weakness and convulsions and usually death in children whose mothers had clinical symptoms of PCT or who had ingested contaminated bread during gestation and/or lactation. The presence of hexachlorobenzene in the milk of nursing mothers suggested that pink sore was a manifestation of hexachlorobenzene toxicity. The reviewer states that there was a 95% mortality in these infants in addition to the very high incidence of stillbirths.

In a follow-up study, Cripps et al. (1980) examined 32 patients 20 years after the onset. Porphyrins were determined in urine and stool specimens of 29 patients and clinically significant porphyrin levels were observed in 5 patients. Clinical features such as hyperpigmentation, scarring, pinched facies, hypertrichosis, enlarged thyroid and distinctive arthritis were present in about half of the patients.

A detailed follow-up study was also conducted with 161 Turkish patients 25 years after the initial hexachlorobenzene incident (Peters et al., 1982). The patient group studied included some of the patients previously examined (Peters et al., 1966). Twenty-six patients were over 17 years of age at the time of acute toxicity, whereas the average age of the remaining patients was 7.1 years. An evaluation of the clinical signs and symptoms is summarized in Table 12-11.

The chronic disease state was manifested by generalized hyperpigmentation and hypertrichosis, scarring on the cheeks and hands, and tight sclerodermoid changes of the nose with perioral scarring. The most striking clinical features in those patients who developed signs of hexachlorobenzene toxicity at an average age of 7 years consisted of painless arthritic changes with osteoporosis of carpal, metacarpal and phalangeal bones and atrophy or failure to develop in the terminal phalanges. In addition, neurologic symptoms including weakness, paresthesias, myotonia, cogwheeling and painless arthritic changes of the hands and feet, were observed in 50-70% of the patients examined. Since the signs and symptoms 20-25 years later represented a continuum of signs and symptoms observed personally by Peters and Gocmen (1959-1963), it was concluded that the symptoms represented the effects of both hexachlorobenzene toxicity and changes caused by the induced mixed porphyria. Control patients from the villages inhabited by these patients included unaffected family members and clearly demonstrated clearly the uniqueness of this disorder which allowed for ready identification of affected patients. In addition the 60% incidence of large thyroid tumors in the females proved a sharp contrast to the 5% incidence of thyroid tumors in the geographical area. No conclusions were drawn as to

TABLE 12-11

Clinical Signs and Symptoms in Humans 25 Years After Exposure to Low Levels in HCB in Turkey, 1955-1959[a]

Clinical Signs/Symptoms	No. of Patients with Symptoms[b]	Percent
Porphyria--Neurological		
Weakness	117 (161)	73
Paresthesias	89 (161)	55
Sensory shading	75 (125)	60
Nervousness	39 (60)	65
Myotonia	35 (76)	46
"Cogwheeling"	34 (125)	27
Colic	84 (161)	52
Constipation	31 (161)	19
Recent red urine	17 (161)	11
Enlarged liver	10 (161)	6
Dermatologic		
Hyperpigmentation	125 (161)	78
Scarring	134 (161)	83
Hirsutism	81 (161)	50
Pinched facies	69 (161)	43
Fragile skin	62 (161)	39
Thyroid enlargement		
Total	64 (161)	40
Men	26 (98)	27
Women	38 (63)	60
Orthopedic and others		
Arthritis	108 (161)	67
Small hands	107 (161)	67
Short stature	74 (161)	46

[a] Source: Peters et al., 1982

[b] Numbers in parentheses represent total number of patients examined for this symptom

HCB = Hexachlorobenzene

the incidence of cancer and mortality. Studies on these endpoints are still in progress and the length of time that has elapsed from the time of exposure may not yet be adequate for drawing conclusions.

A boy and three women treated in the early 1960's with i.v. and/or oral edetic acid (the metal chelating agent EDTA) showed no active symptoms when examined, and skin pigmentation and scarring were much less severe than in most of the other patients. Urine and/or stool porphyrin studies showed that seven patients had clearly recognizable increases in porphyrin levels (Table 12-12). Clinical chemistry and milk residue data are summarized in Table 12-13. Percent δ-aminolevulinic acid values were found to be above the upper normal limit of 4 mg/ℓ in 32 of 55 patients. The average residue levels in human milk samples from Turkish mothers with porphyria was 0.51±0.75 ppm; 0.16±0.23 ppm was found in milk samples from nonporphyric but hexachlorobenzene-exposed mothers.

12.2.3. **Summary.** A few epidemiologic studies with occupationally-exposed workers have been reported, together with studies and surveys conducted in Turkey and in the United States (i.e., Louisana), on the general population following accidental exposure to hexachlorobenzene. These studies qualitatively support the toxicity of hexachlorobenzene but give little dose response information. Biological monitoring of plasma levels show clearly more hexachlorobenzene in plasma of exposed compared to non-exposed individuals although no biologically significant adverse health effects were seen during the observation periods.

The exposure of humans to hexachlorobenzene in Turkey from 1955-1959 caused an epidemic of hexachlorobenzene induced PCT, also known as porphrya turcica, which is manifested by disturbed porphyrin metabolism, cutaneous

TABLE 12-12

Porphyrin Levels in Patients and Controls*

	Stool (µg/g dry weight			Urine (µg/ℓ)	
	Coproporphyrin	Protoporphyrin	Uroporphyrin	Coproporphyrin	Uroporphyrin
Controls					
Turkey, mean ± SD (N=33)	4.80 ± 3.2	7.65 ± 9.83	1.41 ± 1.57	30.0 ± 23.6	5.80 ± 4.25
United States, mean ± SD (N=40)	6.1 ± 4.7	21.1 ± 11.6	2.8 ± 2.7	69.0 ± 27.0	9.0 ± 4.0
Hexachlorobenzene-Exposed Patients					
Patients with active porphyria (N=15)	70.14 (1.0-837.6)	12.19 (0.7-61.8)	25.8 (0.7-189.2)	174.5 (32.6-779.3)	111.4 (16-1607)
Remainder (N=146)	5.74 (0.5-4.1)	9.02 (0-103.4)	1.19 (0-12.6)	31.91 (0-198.4)	7.25 (0-29.5)

*Source: Peters et al., 1982

TABLE 12-13

Laboratory Test Results of Turkish Patients[a]

Test	Normal Range	Patient Range	No. of Abnormal Results[b]
Urine			
δ-Aminolevulinic acid, mg/ℓ	<4	0.14-10.1	32 (55)
Porphobilinogen, mg/ℓ	<1	0.11-1.04	0 (56)
Copper, ppm	0.01-0.06	0.01-0.046	0 (31)
Zinc, ppm	0.1-0.7	0.02-1.22	7 (31)
Serum			
Copper, µg/dℓ	70-155	88-153	0 (30)
Zinc, µg/dℓ	70-120	57-112	9 (29)
Creatine kinase, units/ℓ	women, <120	65-141	1 (8)
	men, <150	51-318	4 (11)
Iron, µg/dℓ	65-170	69-147	0 (29)
Thyroid function tests	5-11	2.2-10.1	women, 5 (10)
Thyroxine, µg/dℓ			men, 2 (9)
Triiodothyronine uptake, percent	37-59	36-51.1	women, 1 (10) men, 1 (9)
Free thyroxine index	1.85-6.5	0.9-4.6	women, 4 (10) men, 0 (9)
Blood			
Lead, erythrocyte, µg/dℓ	<35	2-17	0 (11)
Uroporphyrinogen synthetase[c]	>20	12.4-34.8	5 (30)
Milk hexachlorobenzene, ppm[d]			
Patients with porphyria	NA	0.51 (0-3.12)	53 (56)
Patients without porphyria	NA	0.16 (0-1.26)	16 (77)

[a] Source: Peters, et al., 1982

[b] Numbers in parentheses represent total number of patient specimens analyzed.

[c] Values expressed in nanomoles formed per milliliter of RBCs per hour

[d] Allowable limit in United States for cow's milk is 0.02 ppm

NA = Not applicable

lesions and hyperpigmentation. The authors estimated that 0.05-0.2 g/day were ingested. In children under 1 year of age, pink sore was observed as well as 95% mortality in these infants.

Follow-up studies conducted with patients 20-25 years after the onset of porphyria showed that a few patients still had active porphyria, whereas >50% exhibited hyperpigmentation scarring as well as other dermatologic, neurologic and skeletal features of hexachlorobenzene toxicity. Hexachlorobenzene residues were also found in the blood, fat or breast milk of some patients.

A correlation was found between hexachlorobenzene levels in blood and the number of years worked in a chlorinated solvents plant. The concentration of urinary uroporphyrins and coproporphyrins in workers ranged from 21-37 and 67-101 µg/ℓ, respectively, for the period between 1974 and 1977. An epidemiologic survey conducted with 86 residents in the vicinity of this chlorinated solvents plant showed elevated hexachlorobenzene residues in plasma. Higher levels of hexachlorobenzene residues were found in males than in females, but these were not associated with race or food consumption.

12.3. MAMMALIAN TOXICOLOGY

12.3.1. Acute Toxicity. Information on the acute toxicity of hexachlorobenzene was limited to oral LD_{50} values determined with a few mammalian species. The following LD_{50} values were reported in the available literature: rats, 3500-10,000 mg/kg; rabbits, 2600 mg/kg; cats, 1700 mg/kg; and mice, 4000 mg/kg (IARC, 1979; NAS, 1977; Sax, 1979).

Graef et al. (1979) reported that hexachlorobenzene blocked the activity of rat hepatic 3-hydroxysteroid dehydrogenase leading to the accumulation of 5β-H-steroids, which are known inducers of porphyrin biosynthesis. Hexachlorobenzene-induced porphyria has also been reported to occur as a result of a deficiency in the uroporphyrinogen decarboxylation process that is catalyzed by porphyrinogen carboxylase. This enzyme is the only one in the heme pathway that exhibits a decrease in activity. The inhibition of porphyrinogen carboxylase in liver homogenates from female Wistar rats with severe porphyria induced by hexachlorobenzene was studied by Rios de Molina et al. (1980). Hexachlorobenzene had no effect on enzyme activity at 10^{-3} M, whereas pentachlorophenol caused a 90% inhibition at the same concentration. However, pentachlorophenol did not inhibit the enzyme at a concentration of 10^{-5} M. It was concluded that a concentration $>10^{-5}$ M of pentachlorophenol, possibly together with other hexachlorobenzene metabolites, was needed to cause enzyme inhibition.

Hexachlorobenzene has also been reported to induce the activity of hepatic microsomal enzymes in male or female rats following subchronic administration (Carlson, 1978; Carlson and Tardiff, 1976; Chadwick et al., 1977). Hexachlorobenzene produced a so-called "mixed-type" induction of cytochrome P-450 content in female rats resembling that produced by a combination of phenobarbital (cytochrome P-450) and 3,4-benzopyrene (cytochrome P-448) (Goldstein et al., 1982; Debets et al., 1980a). In female rats, hexachlorobenzene increased the activities of δ-aminolevulinic acid synthetase and aminopyrine demethylase (Ariyoshi et al., 1974), ethoxyresorufin-O-deethylase, aminopyrine demethylase, aryl hydrocarbon hydroxylase, p-nitrophenol glucuronyl transferase, and NADPH-cytochrome c reductase (Goldstein et al., 1982; Debets et al., 1980a). Similarly, in male rats, hexachlorobenzene increased the activities of hepatic ethyl morphine N- and p-nitroanisol O-demethylases, aniline hydroxylase, and UDP glucuronyl transferase (Mehendale et al., 1975), acetanilide hydroxylase, acetanilide esterase, procaine esterase, and arylesterase activities (Carlson et al., 1979; Carlson, 1980).

12.3.2. **Subchronic Toxicity.** Several oral subchronic studies of hexachlorobenzene have been reported, but no studies were located on the effects of hexachlorobenzene following inhalation. In several animal species, hexachlorobenzene was found to cause alopecia and scabbing, decreased body weight, increased liver and kidney weights and increased porphyrin levels in the urine and in several organs. Histopathologic changes were noted in the liver and kidneys of rats, gastric lymphoid tissue of dogs, and ovaries of monkeys. When placed on untreated diets, rats were able to recover from most of the toxic effects of hexachlorobenzene treatment. Hexachlorobenzene was also reported to cause certain neurologic effects (ataxia, paralysis, etc.) on rats, mice, hamsters and female beagles, and to induce an increase in hepatic microsomal enzyme activity. Toxicity data for hexachlorobenzene can be found in Table 12-14.

Iatropoulos et al. (1976) reported that five adult female rhesus monkeys given daily gavage treatments of hexachlorobenzene suspended in 1% carboxymethylcellulose at 8, 32, 64 or 128 mg/kg/day for 60 days, showed extensive morphologic changes in the ovaries. These changes were dose-related.

Subchronic studies conducted by Koss et al. (1980a) with groups of four female rats treated orally (probably by gavage) with 100 mg/kg of hexachlorobenzene in olive oil every other day, suggested that hexachlorobenzene metabolites covalently bind to cytosolic proteins although no binding to uroporphyrinogen decarboxylase was specifically demonstrated.

Elissalde and Clark (1979) reported a significant increase in the *in vitro* metabolism of ^{3}H-testosterone by liver microsomes from male mice fed diets containing 250 mg hexachlorobenzene/kg for 21 days. In addition, decreases in the concentration of testosterone in the serum and in the

TABLE 12-14

Summary of Toxicity Studies on Hexachlorobenzene

Species	Route	Dose	Duration	Effects	Reference
Rat (females)	oral	100 mg/kg every other day	up to 43 days	Suggested covalent binding of hexachlorobenzene metabolites to cytosolic proteins	Koss et al., 1980a
Rat	oral (diet)	0.5 mg/kg/day	15 weeks exposed and held to 48 weeks	Transient increases in liver porphyrin levels in females after termination of exposure	Kuiper-Goodman et al., 1977
		2.0 mg/kg/day	15 weeks exposed and held to 48 weeks	Increases in liver porphyrin levels in females after termination of exposure, increased size of centrilobular hepatocytes	
		8.0 mg/kg/day	15 weeks exposed and held to 48 weeks	Increased liver weights, increased liver, kidney and spleen porphyrin levels in females (porphyria), centrilobular liver lesions especially in females at 48 weeks	
		32.0 mg/kg/day	15 weeks exposed and held to 48 weeks	Increased mortality in females, intension tremors in males and females and ataxia in a few females, increased liver, kidney and spleen weights, increased liver, kidney and spleen porphyrin levels in females (porphyria), centrilobular liver lesions and splenomegaly	
Rat (females)	oral (gavage)	50 mg/kg every other day	15 weeks	Increased liver, kidney, spleen and adrenal weights, porphyria (increased liver porphyrin levels and increased excretion of porphyrins and precursors), tremors, hair loss and skin lesions	Koss et al., 1978b
Rats (females)	oral (gavage)	0.5 mg/kg twice weekly	29 weeks	Increase in relative liver weight	Böger et al., 1979
		2.0 mg/kg twice weekly	29 weeks	Increase in relative liver weight, moderately enlarged hepatocytes	
		8.0 mg/kg twice weekly	29 weeks	Porphyria, markedly enlarged hepatocytes, increase in relative liver weight	
		32.0 mg/kg twice weekly	29 weeks	Porphyria, markedly enlarged hepatocytes, increase in liver weights	
Rat (females)	oral (diet)	100 mg/kg diet	98 days	Porphyria (increased liver lobe porphyrins), decreased activity of uroporphyrinogen decarboxylase	Smith et al., 1980

TABLE 12-14 (cont.)

Species	Route	Dose	Duration	Effects	Reference
Rat	oral (diet and nursing)	50 mg/kg diet	gestation until 5 weeks of age	Depressed resistance to *L. monocytogenes* and *T. spiralis*, enhanced thymus-dependent antibody response	Vos et al., 1979b
		150 mg/kg diet	gestation until 5 weeks of age	Increased serum IgM and IgG, depressed resistance to *L. monocytogenes* and *T. spiralis*, enhanced thymus-dependent antibody response, increased liver and adrenal weights	
Rat	oral (diet)	500, 1000 or 2000 mg/kg diet	3 weeks	Dose-related increases in relative spleen, lymph nodes, liver, adrenals, thyroid, testes and kidney weights, dose-related increase in serum IgM levels, no change in serum IgG levels, dose-related pathological changes in liver, lymph nodes and spleen	Vos et al., 1979a
Rat	oral (diet)	2000 mg/kg diet	10 weeks	Porphyria found microscopically at 5 weeks and grossly at 10 weeks using fluorescence	Gralla et al., 1977
Rat (male)	oral (diet)	2000 mg/kg diet	100 days	Elevated hepatic enzymes by 1 week and increased urinary porphyrin and ALA levels (porphyria) as early as 40 days	Lissner et al., 1975
Rat (female)	oral (diet)	3000 mg/kg diet	11 weeks	Decreased uroporphyrinogen decarboxylase activity and porphyria after 4 weeks	Elder et al., 1976
Rat (female)	oral (gavage)	50, 100 or 200 mg/kg	120 days	Dose- and time-dependent increase in liver and urine porphyrins (porphyria)	Carlson, 1977b
Rat	oral (gavage)	14 mg/kg every other day	103 days	Porphyria in treated females, susceptibility of females to porphyria may be related to estrogen levels	Rizzardini and Smith, 1982
Rat (females)	oral (gavage)	100 mg/kg every other day	6 weeks exposed and held for additional 18 months	Porphyria (liver uroporphyrin levels peaked 7 months postexposure and levels had not returned to normal by 18 months), decreased liver protoporphyrin and coproporphyrin levels, inhibition of uroporphyrinogen decarboxylase activity until 18 months postexposure	Koss et al., 1983
Rat (females)	oral (diet)	6-8 mg/kg/day	75-90 weeks	Decline in body weights, porphyria, enlarged livers and liver tumors	Smith and Cabral, 1980
Rat	oral (diet)	75 mg/kg diet (4-5 mg/kg/day) 150 mg/kg diet (8-9.5 mg/kg/day)	up to 2 years	Porphyria, time-related appearance of severe hepatic and renal pathologies, after 1 year increases in hepatomas, hepatocarcinomas, bile duct adenomas, renal adenomas and renal carcinomas	Lambrecht et al., 1983a,b

TABLE 12-14 (cont.)

Species	Route	Dose	Duration	Effects	Reference
Rat	oral (diet)	0.32, 1.6, 8.0 or 40 mg/kg diet	~130 days	Hematological changes at all dose levels in males, increases in liver and heart weights in males at 8.0 and 40 ppm diets, no treatment-related effects observed in bred females	Arnold et al., 1983
	oral (diet and nursing)	0.32 or 1.6 mg/kg diet	gestation through lifetime (130 weeks)	Glycogen depletion in 1.6 mg/kg males; no effects reported at 0.32 mg/kg	
		8.0 mg/kg diet	gestation through lifetime (130 weeks)	Increase in liver pathologies	
		40 mg/kg diet	gestation through lifetime (130 weeks)	Increased mortality as pups, increase in liver and kidney pathologies, increase in adrenal pheochromocytomas in females and parathyroid tumors in males	
Rat	oral (diet)	10 or 20 mg/kg diet	F_0 to F_4 generations	No effects reported	Grant et al., 1977
		40 mg/kg diet	F_0 to F_4 generations	Increases in liver weights and aniline hydroxylase activity	
		80 mg/kg diet	F_0 to F_4 generations	Decreased body weights, F_3 and F_4 generations had decreased lactation index and postnatal viability and increased stillbirths	
		160 mg/kg diet	F_0 to F_4 generations	Increased mortality and decreased lactation index starting in F_1 generation	
		320 and 640 mg/kg diet	F_0 to F_4 generations	20 and 50% mortality in F_0 320 and 640 mg/kg groups, respectively, greatly reduced fertility index and litter size and increase in stillbirths, viability index zero in F_1	
Rat	oral (diet)	60, 80, 100, 120 or 140 mg/kg diet	F_0 to F_{1a} and F_{1b} generations	Increased mortality in all groups at 21 days, 21-day LD_{50} values for pups were 100 and 140 mg/kg for F_{1a} and F_{1b} generations, respectively	Kitchin et al., 1982
Rat	oral (diet)	0 or 80 mg/kg diet	gestation and nursing or cross nursed with controls	Nursing exposure produced greater effects than did gestational exposure, effects noted were: smaller brains, hearts, kidneys and spleens, increased liver weights	Mendoza et al., 1978
Rat	oral (diet)	80 mg/kg diet	2 weeks prior to mating to 35-36 days after weaning	Increased porphyrin levels and decreased liver esterase activity in dams, no changes in gestation indices or neonatal survival	Mendoza et al., 1979

TABLE 12-14 (cont.)

Species	Route	Dose	Duration	Effects	Reference
Rat	oral (gavage)	10, 20, 40, 60, 80 or 120 mg/kg	days 6-21 of gestation	Maternal toxicity (weight loss, tremors and convulsions) and reduced fetal weights at 120 and 80 mg/kg maternal doses, dose-related increase in incidence of unilateral and bilateral 14th rib, sternal defects were also noted in one experiment	Khera, 1974
Mouse	oral (diet)	2.5, 25 or 250 mg/kg diet	21 days	Dose-related increase in liver and decrease in prostate and seminal vesicle weights, dose-related alterations in testosterone metabolism, altered hepatic enzyme levels	Elissalde and Clark, 1979
Mouse (male)	oral (diet)	10 mg/kg diet (8.4 (mg/mouse/24 weeks) or 50 mg/kg diet (35.3 mg/mouse/ 24 weeks)	24 weeks	Dose-related reduction in weight gain, no tumor pathology observed	Shirai et al., 1978
Mouse (male)	oral (diet)	167 mg/kg diet	3-6 weeks	Impairment in host resistance as measured by increased sensitivity to _S_. _typhosa_ and _P_. _bergheri_, and decrease in IgA levels	Loose et al., 1978a,b
Mouse	oral (diet)	6, 12, 24 and 36* mg/kg/day	101-120 weeks *(15 weeks exposed held until 120 weeks)	Reduced growth rate at all dose levels, shortened lifespan associated with tremors and convulsions in 24 and 36 mg/kg/day groups, dose-dependent increase in liver-cell tumors in the 12, 24 and 36 mg/kg/day dose groups	Cabral et al., 1979
Mouse	oral (gavage)	100 mg/kg/day to pregnant mice	days 7-16 of gestation	Increased maternal livers and decreased fetal body weights, increased incidence of abnormal fetuses per litter observed	Courtney et al., 1976
Hamster	oral (diet)	200 or 400 mg/kg diet	90 days	Precirrhotic and cirrhotic hepatic lesions, bile-duct hyperplasias and hepatomas	Lambrecht et al., 1982
Hamster	oral (diet)	4, 8 or 16 mg/kg/day	lifespan	Shortened lifespan in 16 mg/kg/day group, increase in hepatomas at all dose levels, increase in liver haemangioendothelioma in males and females and an increase in thyroid alveolar adenomas in males in 16 mg/kg/day group	Cabral et al., 1977
Cats (breeding females)	oral (diet)	3 or 8.7 mg/day/cat	142 days	Weight loss and increased disease susceptibility in bred females, dose-related decrease in litter size and survival of offspring, hepatomegaly in offspring	Hansen et al., 1979

TABLE 12-14 (cont.)

Species	Route	Dose	Duration	Effects	Reference
Minks	oral (diet)	1 or 5 mg/kg diet	during gestation until 17 weeks of age	Dose-related increase in offspring mortality, induction of hepatic MFO enzymes in exposed offspring	Rush et al., 1983
Dog (female)	oral (capsule)	50 or 150 mg/kg/day	21 days	Liver and hepatocyte enlargement, dose-induced electroencephalogram dysrhythmias	Sundlof et al., 1981
Dog	oral (capsule)	1, 10, 100 or 1000 mg/day/dog	1 year	Increase in mortality, neutrophilia, and anorexia in the 100 and 1000 mg dose groups, dose-related nodular hyperplasia of gastric lymphoid tissue in all treated animals	Gralla et al., 1977
Monkey (female)	oral (gavage)	8, 32, 64 or 128 mg/kg/day	60 days	Dose-related pathology in liver, kidney, ovaries and thymus	Iatropoulus et al., 1976
Monkey	oral (nursing)	7.51-186 ppm milk	60 days	2 of 3 infants died as a result of exposures	Bailey et al., 1980

weights of seminal vesicles and ventral prostates were reported. Hexachlorobenzene was also reported to cause certain neurologic lesions in male and female rats, hamsters and mice fed diets containing varous levels of hexachlorobenzene for 13 weeks. These included hyperemia, edema, arborization and hemorrhages in the brain and meninges. The lesions extended to the cerebrum, cerebellum, medulla, spinal cord and their meninges. The severity of these lesions was higher in males and was dose dependent in both sexes (Headley et al., 1981). Physiologic changes (electroencephalogram dysrhythmias) in the central nervous system were reported in 10 female beagles receiving gelatin capsules containing doses of 50 or 150 mg/kg of hexachlorobenzene for 21 days (Sundlof et al., 1981).

Kuiper-Goodman et al. (1977) conducted a 15-week subchronic feeding study wherein groups of 70 male and 70 female COBS rats were fed diets providing 0, 0.5, 2, 8 or 32 mg/kg bw/day of hexachlorobenzene dissolved in corn oil (5%). Female rats were more susceptible to hexachlorobenzene than males, as indicated by all the parameters studied, and a NOEL of 0.5 mg/kg/day was suggested by the authors. This NOEL may be better interpreted as a NOAEL since a transient increase in liver porphyrin levels was observed in females 4 weeks after removal from hexachlorobenzene. The 2 mg/kg/day dose may be interpreted as a LOAEL since this level caused increases in liver porphyrin levels in females even 33 weeks after removal from hexachlorobenzene, and increases in the relative observed severity of centrilobular liver lesions as compared to control rats. About 40% mortality occurred in females, but none in males at the highest dose. Clinical signs included intention tremor, excessive irritability, multiple alopecia, scabbing and ataxia, with hind leg paralysis at the highest dose. There was a significant increase in liver and kidney weights at the higher doses. An increase in liver weight was also found in groups of 36 female Wistar rats treated by gavage twice weekly with hexachlorobenzene dissolved in olive oil at 32 mg/kg for 29 weeks (Boger et al., 1979). Similarly, Koss et al. (1978b) reported a 1.5- to 2-fold increase in the weights of the liver, spleen, kidneys and adrenal glands from female Wistar rats treated orally (esophageal tube) with 50 mg/kg of hexachlorobenzene dissolved in corn oil every other day for 15 weeks. When hexachlorobenzene-treated rats were placed on untreated diets, they no longer showed signs of hexachlorobenzene toxicity, such as dermal lesions, and body and organ weights returned to normal (Kuiper-Goodman et al., 1977; Koss et al., 1978b). Enlarged livers were reported in subchronic studies with female beagles (Sundlof et al., 1981) and male mice (Shirai et al., 1978) administered hexachlorobenzene in diet.

A dose-dependent enlargement of hepatocytes was observed in groups of 36 female Wistar rats receiving gavage treatments of olive oil containing hexachlorobenzene (99.8% pure) 0.5, 2.0, 8.0 and 32 mg/kg twice weekly for 29 weeks (Boger et al., 1979). This effect was associated with the proliferation of the smooth endoplasmic reticulum in the centrilobular area, and an increase in glycogen deposits; however, animals receiving 0.5 mg/kg did not develop enlarged hepatocytes. In addition, atypical membrane complexes in treated animals were noted and liver-cell mitochondria were moderately enlarged and had irregular shapes. An increase in the size of centrilobular hepatocytes was also reported in male and female rats receiving 2 mg/kg/day for 15 weeks, together with histopathologic changes in the spleen (Kuiper-Goodman et al., 1977).

Nodular hyperplasia of gastric lymphoid tissue was reported in groups of 6 male and 6 female beagles receiving daily gelatine capsules containing 1, 10, 100 and 1000 mg hexachlorobenzene/dog/day for 12 months (Gralla et al., 1977). Extensive dose-related histopathologic changes were also observed in ovaries from groups of two rhesus monkeys given daily methyl cellulose/distilled water solutions containing doses of 8, 16, 32, 64 or 128 mg hexachlorobenzene/kg of body weight by gavage for 60 days (Knauf and Hobson, 1979; Iatropoulas et al., 1976). Shirai et al. (1978) conducted a 24-week study with male mice fed diets containing 10 or 50 ppm of hexachlorobenzene, followed by a recovery period of 14 weeks. Histologic examination revealed no pathologic changes in the liver or any other organ.

Lambrecht et al. (1982) fed male and female Syrian golden hamsters hexachlorobenzene at doses of 0, 200 and 400 ppm in their diet for 90 days. The hamsters were killed on day 91 and at 6-week intervals through day 361. No differences were seen in growth and food consumption between control and exposed animals. The liver was reported as the most severely affected organ exhibiting a variety of precirrhotic and cirrhotic lesions, bile-duct hyperplasias and hepatomas. The incidence of neoplasms found in this study will be further discussed in Section 12.3.5.

Hexachlorobenzene has been found to cause increased porphyrin levels in the liver of male and female rats receiving the compound incorporated into the diet at doses of 8 and 32 mg/kg/day for 15 weeks (Kuiper-Goodman et al., 1977). Koss et al. (1978b) reported that female rats treated orally with 50 mg hexachlorobenzene/kg every other day for 15 weeks still showed increased levels of porphyrin in the liver, 38 weeks after the last treatment. In addition, porphyrin, δ-aminolevulinic acid, and porphobilinogen levels in the urine gradually increased during the 15-week treatment period, but subsequently decreased to normal levels. Smith et al. (1980) reported that the lobes of livers from female Agus rats fed diets containing 0.01% hexachlorobenzene developed porphyria at different rates. During the initial course of treatment, porphyria in the caudate lobe developed at a significantly slower rate than the median, left or right sections of the liver, but eventually, all lobes became equally porphyric. In contrast, porphyria was not observed when viewed for hepatic fluorescence of porphyrins in male and female beagle dogs treated daily with 0, 1, 10, 100 or 1000 mg/dog/day for 1 year (Gralla et al., 1977). Gralla et al. (1977) observed that female CD rats fed 0.2% hexachlorobenzene were porphyric using this fluorescence method.

Rizzardini and Smith (1982) clearly confirmed that female rats are more susceptible to hexachlorobenzene-induced porphyria than are male rats, and that this difference in susceptibility is probably associated with the faster metabolism of hexachlorobenzene in females. They intubated male and female F344/N rats every other day for 103 days with 14 mg/kg (50 μmole/kg) hexachlorobenzene dissolved in arachis oil and monitored the rats for hexachlorobenzene metabolites and porphyrin levels. The results indicated that after 75 days of hexachlorobenzene treatment the excretion of urinary porphyrins increased rapidly in the females and after 103 days the females had urine and liver porphyrin levels 40- and 310-fold higher, respectively, than did the males. During this treatment period the females were found to excrete greater quantities of hexachlorobenzene metabolites, especially pentachlorothiophenol, than the males. Estrogen levels seem to play an important part in the increased susceptibility of females to hexachlorobenzene-induced porphyria. When both male and female rats were pretreated intraperitoneally with four doses of 20 μmole/kg of diethylstilboestrol dipropionate (an estrogenic drug), both sexes had stimulated excretion of hexachlorobenzene metabolites.

A better understanding of hexachlorobenzene-induced porphyria was provided by Koss et al. (1983). These researchers administered every other day for 6 weeks, through stomach tube, 100 mg/kg hexachlorobenzene dissolved in olive oil to female Wistar rats and then observed the rats for an additional 18 months. The rats were evaluated during both the exposure period and the 18-month holding period for liver hexachlorobenzene levels, levels of liver porphyrins, and the activity of liver uroporphyrinogen decarboxylase. The results revealed a rapid increase in hexachlorobenzene liver levels which reached a plateau after 10 days of treatment and remained constant until exposure was terminated at 6 weeks. The levels of liver hexachlorobenzene then decreased over time with no valid biological halflife determinable. The liver porphyrin levels, however, started to rise slightly after 3 weeks of hexachlorobenzene exposure and reached a maximum liver porphyrin concentration ~7 months after the exposures had ceased (Table 12-15). The liver porphyrin levels decreased to a constant level ~14 months after ceasing hexachlorobenzene exposures. At 18 months after ceasing exposures, the treated rats liver porphyrin levels were still substantially higher than the levels in control rats. The distribution pattern of the liver porphyrins was observed to be changed as early as after the second hexachlorobenzene administration. The observed changes were increases in liver uroporphyrin levels and decreases in liver protoporphyrin and coproporphyrin levels. The change in porphyrin patterns was traced to the decreased activity of uroporphyrinogen decarboxylase activity which was found to be not detectable at the end of the 6-week exposure period and the activity did not become detectable again until 18 months postexposure

TABLE 12-15

Porphyrin Content and Uroporphyrinogen Decarboxylase Activity in the Liver Cytosol of Female Rats Pretreated with 100 mg/kg HCB Every Other Day for 6 Weeks[a]

Time After the End of Treatment	Porphyrin Content (nmol/6 mℓ cytosol)[b]	Enzyme Activity (pmol · mg^{-1} · min^{-1})[c]
1 day	14 ± 3[d]	ND[e]
7 months	133 ± 15	ND
14 months	9 ± 6	ND
18 months	8 ± 5	0.3 ± 0.2[d]
Controls	0.06 ± 0.04	0.5 ± 0.1

[a] Source: Koss et al., 1983

[b] 6 mℓ cytosol correspond with 1 g liver tissue

[c] pmol coproporphyrinogen I (determined as coproporphyrin) formed from uroporphyrinogen I in 1 min by 1 mg cytosol protein

[d] Mean (± SD) of three or four animals

[e] ND = Not detectable. The lower detection limit was determined at 0.02 pmol · mg^{-1} · min^{-1} coproporphyrin

HCB = Hexachlorobenzene

(see Table 12-15). These data led the investigators (Koss et al., 1983) to propose that there are four phases of hexachlorobenzene-induced porphyria:

> During the first phase an almost constant content of hexachlorobenzene and a gradual decrease of uroporphyrinogen decarboxylase activity is achieved. In the second phase a noticeable accumulation of porphyrins and a practically complete inhibition of decarboxylase activity are conspicuous. In the third phase, which occurs after hexachlorobenzene administration has been discontinued, a further accumulation of porphyrins and a continuing inhibition of uroporphyrinogen decarboxylase activity can be seen, even after extensive elimination of hexachlorobenzene. During the fourth phase a decrease in porphyrin content and a return of decarboxylase activity are clearly observable.

A possible reason for the continued inhibition of uroporphyrinogen decarboxylase activity, even after substantial elimination of hexachlorobenzene has occurred, was also discussed in this report. Koss et al. (1983) presented the scenario that once hexachlorobenzene had caused an inhibition of uroporphyrinogen decarboxylase activity and increased liver porphyrin levels that the accumulation of porphyrins could themselves maintain the inhibition of the enzyme activity.

Hexachlorobenzene pretreatment has been reported to cause altered immune responses. Vos et al. (1979b) studied the effect of hexachlorobenzene on the immune system after combined pre- and postnatal exposure. Wistar rats were fed diets containing 50 or 150 μg/kg hexachlorobenzene during pregnancy and lactation. The pups were weaned after 3 weeks and continued on the test diets until 5 weeks of age, when their immune system was functionally assessed. At the higher dietary level, hexachlorobenzene caused a statistically signficant increase in serum IgM and IgG concentrations.

Hexachlorobenzene treatment also caused a decreased resistance to infection with Listeria monocytogenes (Vos et al., 1979b). The LD_{50} values were reported to be 14×10^5, 7.1×10^5 and 5.0×10^5 bacteria in pregnant Wistar rats receiving diets containing 0, 50 and 150 mg/kg, respectively.

Similarly, decreased resistance of Trichinella spiralis infection, as indicated by an increase in the number of larvae found in muscle tissue, was noted. Hexachlorobenzene also enhanced the thymus-dependent antibody response to T. spiralis antigen and to tetanus toxoid. No effects were observed on allograft rejection, mitogenic response of thymus and spleen cells, thymus-independent IgM response to Escherichia coli lipopolysaccharide, passive cutaneous anaphylaxis, and on the clearance of carbon particles and L. monocytogenes. The authors concluded that hexachlorobenzene suppressed cellular immunity and enhanced humoral immunity in both test groups.

In contrast, hexachlorobenzene pretreatment of weanling rats did not alter their cell-mediated immunity, but did stimulate their humoral immune response and enhanced the in vitro responsiveness of spleen cells to different mitogens, which was mainly a result of an increase in the number of splenic lymphocytes. The rats received diets containing 1000 μg hexachlorobenzene/g for 3 weeks after weaning, before assessing their immune system (Vos et al., 1979a).

Loose et al. (1978a,b) found that hexachlorobenzene pretreatment also resulted in impaired host resistance. Male BALB/c mice received diets containing 167 μg hexachlorobenzene/g for 3 or 6 weeks before assessing their immune functions. The concentration of IgA was significantly decreased, whereas those of IgG and IgM did not exhibit consistent significant alterations as compared with the controls. Hexachlorobenzene-treated mice were more sensitive to gram-negative endotoxin (Salmonella typhosa), showed a decreased resistance to a malaria challenge (Plasmodium berghei), and exhibited signficantly depressed antibody synthesis.

12.3.3. **Chronic Toxicity.** Cabral et al. (1977) studied the tumorigenicity of hexachlorobenzene in 6-week-old Syrian golden hamsters given 0, 50 (4 mg/kg/day), 100 (8 mg/kg/day) and 200 (16 mg/kg/day) ppm hexachlorobenzene in their diets for their remaining lifespan. Shortened lifespan was observed in the male and female 200 ppm dose groups after 70 weeks of exposure along with marked weight reduction in the males. Neoplasms were increased by the hexachlorobenzene exposures and are reported in Section 12.3.5. No other pathologies were reported in this study.

Cabral et al. (1979) studied the tumorigenicity of 6- to 7-week-old male and female outbred Swiss mice given 0, 50 (6 mg/kg/day) 100 (12 mg/kg/day) and 200 (24 mg/kg/day) ppm hexachlorobenzene for 101-120 weeks and 300 ppm (36 mg/kg/day) hexachlorobenzene for 15 weeks and held until 120 weeks of age. Results indicated that shortened lifespan occurred in the 200 and 300 ppm dose groups starting after the 30th week of the test and that this reduced survival was associated with tremors and convulsions. Reduction in the rate of growth was observed in female mice in the 50, 200 and 300 ppm dose groups and more pronounced growth rate reduction was observed in male mice in the 100, 200 and 300 ppm dose groups. An increase in neoplasms were found as a result of hexachlorobenzene exposures and are discussed in Section 12.3.5. No other pathologies were reported in this study.

Smith and Cabral (1980) fed young female Agus or MRC Wistar rats 100 ppm (6-8 mg/kg/day) hexachlorobenzene in a diet containing 2% arachis oil for 90 weeks. Hexachlorobenzene exposure resulted in a steady decline in body weights over the study period and in the exposed rats possessing less hair than the controls. Tremors or other nervous symptoms were not seen during this study. Onset of porphyria was observed in the hexachlorobenzene rats after ~3 months, as indicated by urines fluorescing red under UV light, and liver porphyria was confirmed at autopsy by a red fluorescence of the liver. The livers were enlarged 2-fold in the hexachlorobenzene-exposed females and were associated with multiple liver cell tumors. This neoplastic incidence will be discussed in Section 12.3.5.

Male and female Sprague-Dawley rats were fed hexachlorobenzene diets for 2 years containing 0, 75 or 150 ppm hexachlorobenzene (Lambrecht et al., 1983a,b). Four rats per group were killed at weeks 0, 1, 2, 3, 4, 8, 16, 32, 48 and 64 of the study and liver and kidney evaluations were made. Times of appearance of lesions were as follows: 4 weeks -- hepatic hyperemia, edema, parenchymal and hydropic degeneration, renal hyperemia, congestion, swelling and parenchymal degenerations; 32 weeks -- renal tubular nephritis with hyaline casts, severe parenchymal degeneration, epithelial necrosis accompanied by proximal convoluted tubular regeneration, and preneoplastic foci; and 36 weeks -- hepatic preneoplastic foci; and 64 weeks -- hepatic neoplasms and renal neoplasms. The incidence of neoplasms will be further discussed in Section 12.3.5.

A two-generation hexachlorobenzene (analytical grade) feeding study was conducted using Sprague-Dawley rats fed diets containing 0 (64 males, 64 females), 0.32 (40 males, 40 females), 1.6 (40 males, 40 females), 8.0 (40 males, 40 females), or 40.0 (66 males, 66 females) ppm hexachlorobenzene (Arnold et al., 1983). The parental rats (F_0) received their respective test diets for 90 days before mating and until 21 days after parturition (at weaning), at which time they were killed and evaluated for hexachlorobenzene-induced effects. The number of offspring (F_1 generation) from these matings were reduced to 50 males and 50 females per dose group at 28 days of age and fed their respective parents' diets. Thus, the F_1 animals were exposed to hexachlorobenzene and metabolites _in utero_, from maternal nursing and from their diets for the remainder of their lifetime (130 weeks).

The results from this two-generation study indicated no consistent treatment-related effects upon growth or food consumption in either generation and no change in fertility, gestation or lactation indices. A decreased viability index was noted in the 40.0 ppm group relative to controls. No treatment-related effects were found in the F_0 females. The F_0 males were found to have significantly increased liver, heart and brain weights in the 8.0 ppm group and significantly increased liver and heart weights in the 40.0 ppm group. The F_0 males were observed to have various significant changes in hematological parameters at all dose levels. Neoplasms were seen in the F_1 generation and are discussed in Section 12.3.5. The F_1 generation had dose-related significant increases in: 1) centrilobular basophilic chromogenesis reported as slight in the 8.0 and 40.0 ppm male and female dose groups and as moderate in the 40.0 ppm male and female dose groups; 2) peribiliary lymphocytosis and fibrosis in the 40.0 ppm male group; and 3) severe chronic nephrosis in the 40.0 ppm male dose group.

In a second study conducted by Arnold et al. (1983), 50 male Sprague-Dawley rats per group were fed hexachlorobenzene (0 or 40 ppm) and various levels of vitamin A diet (0.1, 1 or 10 times normal control levels). The test groups were as follows: control diet; control diet plus 40 ppm hexachlorobenzene; 1/10 vitamin A diet; 1/10 vitamin A diet plus 40 ppm hexachlorobenzene, 10 times control vitamin A diet; and 10 times vitamin A diet plus 40 ppm hexachlorobenzene. Five rats per group were killed and evaluated both at 25 and 49 weeks and the remaining animals were killed and evaluated after 119 weeks.

Results revealed that the animals on the 1/10 vitamin A diet had significantly reduced body weights and survivability when compared with control diet animals. The animals on 1/10 vitamin A plus 40 ppm hexachlorobenzene diet had significantly decreased body weights and did not survive as long as rats receiving the control diet plus 40 ppm hexachlorobenzene. Hematological evaluations revealed no consistent treatment-related effects. Neoplasms were observed in the test animals and are discussed in Section 12.3.5. No significant differences were found in the incidence of any pathological lesions between the test groups.

12.3.4. Mutagenicity. In a dominant lethal mutation study, male rats (strain not given) received by gavage 0, 70 or 221 mg hexachlorobenzene/kg body weight orally for 5 consecutive days. A dose-dependent reduction in male reproductive performance was observed, but hexachlorobenzene did not induce dominant lethal mutations (Simon et al., 1979). Khera (1974) also reported a lack of dominant lethal mutations in Wistar rats following oral administration of 0, 20, 40 or 60 mg hexachlorobenzene/kg for 10 consecutive days. In 14 sequential mating trials, no significant differences in the incidence of pregnancies, corpora lutea, live implants and deciduomas between the treated and control groups were observed. Mutagenic activity has been observed in a yeast, Saccharomyces cerevisiae, assay (Guerzoni et al., 1976). The mutagenicity of hexachlorobenzene was investigated in three strains of S. cerevisiae using reversion from histidine and methionine auxotrophy, and hexachlorobenzene was reported to be mutagenic at a minimum concentration of 100 ppm.

Lawlor et al. (1979) measured the activity of hexachlorobenzene in the Ames assay, strains TA98, TA100, TA1535, TA1537 and TA1538, at five unspecified dose levels both with and without metabolic acitivation by Aroclor 1254 induced rat liver microsomes. Hexachlorobenzene possessed no detectable levels of mutagenic activity in any of the Salmonella strains used either with or without microsomal activation. These results were reported in an abstract with few experimental details. In addition, this result is not unexpected because the Salmonella test system is generally insensitive to chlorinated compounds.

12.3.5. **Carcinogenicity.** Studies on the carcinogenic potential of hexachlorobenzene have been carried out on hamsters, mice and rats.

12.3.5.1 HAMSTER STUDIES --

12.3.5.1.1. **Cabral et al. (1977)** -- In one study on Syrian golden hamsters (Cabral et al., 1977) hexachlorobenzene was administered in the diet at 50, 100 or 200 ppm. These concentrations correspond to dosages of 4, 8 and 16 mg/kg/day based on body weight and food intake averages. The hexachlorobenzene was prepared by dissolution in corn oil which was then mixed with the feed. The feed was analyzed periodically to insure that the intended level of hexachlorobenzene was maintained (Mollner, 1983). The hexachlorobenzene preparation used in this study was 99.5% pure. Impurities reported to be present in some hexachlorobenzene preparations include chlorinated dibenzofuran and chlorinated dibenzo-p-dioxin, both members of classes of compounds which are carcinogens (Villeneuve et al., 1974). The dosages selected for this study were chosen in order to be comparable to those believed to be consumed by victims of accidental hexachlorobenzene ingestion in Turkey.

In this study on hamsters it was difficult to determine from the published report whether an MTD was reached or exceeded because the information on mortality and weight changes was not detailed enough for unambiguous evaluation. Although mortality was monitored, the investigators only stated that 71% of the treated animals were alive at 50 weeks and that at the highest dose, 16 mg/kg bw/day, there was a reduced lifespan among treated animals after 70 weeks. The study was run for the lifetime of the animals, but the actual duration in weeks was not given. Since the investigators also reported "marked weight reduction" in the highest dose group one could conclude that the MTD may have been reached. However, in the absence of weight data definite conclusions cannot be made.

The tumor incidence among the hamsters is given in Table 12-16. The incidence of hepatomas in males and females was statistically significant in all treated groups. The incidence of liver haemangioendothelioma in males and females was statistically significant in the high dose groups and in males in the middle dose groups. There was a significant dose-related trend for both tumor types. Three instances of metastases were found among the animals with liver haemangioendothelioma. No hepatoma metastases were found. One of the hepatomas in a female animal was found at necropsy at 18 weeks; the investigators did not indicate which dosage level this animal received.

Hamsters in the control groups showed no thyroid tumors but thyroid alveolar adenomas were significantly increased in the high dose males and there was a significant dose-related trend. In female thyroid tumors occurred in all treated groups of females but were not statistically significant.

Chemical induction of thyroid tumors has not been identified with chemically related compounds except for toxaphene, which is a mixture of chlorinated camphene derivatives. Other chemicals associated with induction of thyroid tumors are thioureas, thiouracils, 3-amino-4-ethoxyacetanilide, amitrok, o-anisidine, 2,4-diaminanisole sulfate, ethionamide, 4,4'-methylene

TABLE 12-16

Tumor Incidence in Hamsters Given HCB in the Diet*

Group	Effective No.	TBA No.	%	No. of Tumors		More Than One Tumor		Thyroid		Hepatoma		Haemangioendotheliomas				Other	
												Liver		Spleen			
				No.	per Hamster	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Control	39 F	5	12.8	5	0.13	0	0	0	0	0	0	0	0	1	2.5	4	10.2
	40 M	3	7.5	3	0.08	0	0	0	0	0	0	0	0	0	0	3	7.5
50 ppm (4 mg/kg)	30 F	16	53.3	21	0.70	4	13.3	2	6.6	14	46.6	0	0	0	0	5	16.6
	30 M	18	60.0	27	0.90	8	26.6	0	0	14	46.6	1	3.3	1	3.3	11	36.6
100 ppm (8 mg/kg)	30 F	18	60.0	32	1.06	11	36.6	1	3.3	17	56.6	2	6.6	3	10.0	9	30.0
	30 M	27	90.0	45	1.50	14	46.6	1	3.3	26	86.6	6	20.0	3	10.0	9	30.0
200 ppm (16 mg/kg)	60 F	52	86.6	73	1.21	15	25.0	3	5.0	51	85.0	7	11.6	4	6.6	8	13.3
	57 M	56	98.2	87	1.52	27	47.3	8	14.0	49	85.9	20	35.0	4	7.0	6	10.5

*Source: Cabral et al., 1977

TBA = Tumor-bearing animals

HCB = Hexachlorobenzene

bis(n,n'-dimethyl) n,n'-dimethylbenzenamine, 1,5-naphthylenediamine, 4,4'-oxydianaline, pronetalol•HCl, 4,4'-thiodianaline, iodoform, dibromomethane and dichloroethane (Kraybill, 1983; Weisburger, 1983). Hexachlorobenzene is in a different chemical class from these agents.

Induction of thyroid tumors in the animal studies is of particular interest because a very high incidence of thyroid "tumors" (most of which have not been examined histologically as a result of surgery or biopsy) is found among victims of accidental exposure to hexachlorobenzene in Turkey (Peters, 1983). The incidence among females, over 25 years after the incident, is 61.4% whereas the background incidence in that geographic area for females is about 5% (Peters, 1983). The data and pathology reports have not been made available yet, but it is clear that the cohort exposed to hexachlorobenzene has an unexpectedly high incidence of enlarged thyroid.

While the average dosages used in the study were 4, 8 or 16 mg/kg/day the effective doses are likely to be lower. A number of studies on the absorption of hexachlorobenzene have been conducted (Albro and Thomas, 1974; Mehendale et al., 1975; Koss and Koransky, 1975; Ingebrigsten et al., 1981) which indicate that in an appropriate solvent, such as corn oil, absorption is on the order of 70-80%. Further, hexachlorobenzene distributes itself to various tissue compartments unevenly. Tables 12-17 and 12-18 show the tissue distribution for rats. Zabik and Schemmel (1980) reported that the composition of the diet affects the tissue storage of hexachlorobenzene. It is reasonable to assume that in this hamster study of Cabral et al. (1977) portions of the absorbed dose were sequestered in the fat and mobilized only when fat stores were utilized (i.e., if the animals lost weight). Indeed Villeneuve and Newsome (1975) have shown that when rats were fed hexachloro-

TABLE 12-17

HCB Levels in Tissues of Male Rats Following Administration of 8 mg/kg in Sunflower Oil for 19 Days*

Tissue	HCB Level
Fat tissue	82 ppm
Muscle	17 ppm
Liver	125 μg
Kidneys	12 μg
Spleen	9 μg
Heart	1.5 μg

*Source: Engst et al., 1976

HCB = Hexachlorobenzene

TABLE 12-18

HCB Levels in Tissues of Male Rats Following Administration of ^{14}C-HCB in Arachis Oil. Measurement 7 Days After Single Dose of 5 mg/kg[a]

Organ or Tissue	% Total Radioactivity Administered
Fat[b]	42.81 ± 6.14
Muscle[c]	9.41 ± 1.17
Skin[d]	8.64 ± 1.21
Liver	3.01 ± 0.23
Small intestine	2.43 ± 0.47
Bone[e]	1.04 ± 0.09
Kidneys	0.76 ± 0.11
Large intestine	0.43 ± 0.08
Stomach	0.36 ± 0.04
Blood	0.24 ± 0.04
Lungs	0.24 ± 0.04
Testes	0.21 ± 0.04
Heart	0.18 ± 0.03
Brain	0.17 ± 0.03
Spleen	0.04 ± 0.002
Total in tissues	70.09 ± 5.48
Excretion	
Feces	16.02 ± 2.31[f]
Urine	0.85 ± 0.13[f]
Gut contents	2.48 ± 0.45
Total recovery	89.44 ± 10.57

[a] Source: Mehendale et al., 1975

[b] Based on 9% body weight as fat

[c] Based on 50% body weight as muscle

[d] Based on 16% body weight as skin

[e] Based on 10% body weight as bone

[f] Cumulative total for 7 days

HCB = Hexachlorobenzene

benzene followed by a normal intake of hexachlorobenzene-free diet, hexachlorobenzene was not mobilized from the fat stores. If, however, the hexachlorobenzene-containing diet was followed by an hexachlorobenzene diet which was restricted in calories, causing the animals to mobilize their fat stores, then the stored hexachlorobenzene was mobilized and redistributed to other tissues as the fat stores were utilized. The effective dose of hexachlorobenzene will, therefore, be expected to vary in a chronic hexachlorobenzene study depending upon whether hexachlorobenzene has accumulated in existing fat and remains there as a sink, or whether such stores are mobilized, and at what rates. In the early stages of treatment, when the animals are growing, if the dose is not adjusted to body weight, the effective dose will reflect the greater proportional food intake/body weight characteristic of the growing animal. Later the effective dose could be higher than the administered level because it will consist of the amount taken in as well as the amount released from sequestered stores. These mechanisms should apply to hamsters as well as to the rats.

Since Cabral et al. (1977) did not report the actual weight data in their hamster study and since accompanying pharmacokinetic data are not available, it is not possible to make quantitative evaluation of the hexachlorobenzene contribution to, or from, stored compartments in order to determine effective dose. However, consideration of the absorption factors, and on the assumption that for a major part of the treated animal's life it deposited some of the administered hexachlorobenzene in fat stores, it is reasonable to conclude that the effective dose will be somewhat below the administered dose. If a figure of 75% absorption is used, then the lowest dose used in the study, 4 mg/kg bw/day, is effectively closer to 3 mg/kg bw/day. At that dosage the incidence of hepatoma was 47% and the incidence of thyroid adenomas was 7% among female hamsters, and at this same dose in males the hepatoma incidence was 47% and the incidence of liver haemangioendothelioma was 3%. Unfortunately, information is not available about the total number of weeks on test, or the time of death for the animals with tumors, so that total doses cannot be calculated from information supplied in the published report. These facts would be useful in calculation of potency of hexachlorobenzene in hamsters.

This hamster study provides strong positive evidence of tumorigenicity and evidence of carcinogenicity of hexachlorobenzene, as indicated by the significant increase in hepatomas, significant increase of thyroid adenomas in males and the occurrence of metastasizing liver haemangioendotheliomas in treated but not in control animals. Although not reported in detail in this one page publication, the authors noted an increase in adrenal neoplasms as well. The data presented show that the tumor incidence is positively dose-dependent in most instances and that this is true not only of the number of animals with tumors of all sites but also for the number of tumors per animal. The authors also indicated that latency period was reduced, but actual supporting data was not presented.

12.3.5.1.2. **Lambrecht et al. (1982a) Hamster Study** -- Another study on hamsters, carried out in a different laboratory, adds further suggestive evidence for the tumorigenicity of hexachlorobenzene in hamsters (Lambrecht et al., 1982a). This study, reported only in abstract form, was also carried out in the Syrian golden hamster. In this study the animals were exposed for only 90 days to the hexachlorobenzene. On day 91, half of the initial exposed 50 animals were sacrificed. The remaining animals were sacrificed periodically until the end of the 1-year study. The exposure levels used were 200 or 400 ppm hexachlorobenzene in the diet. Assuming that the hamsters from the Cabral (1977) study were comparable in weight and dietary consumption, these ppm figures would be approximately equal to and twice those of the high dose used in the lifetime studies of Cabral et al., 1977). Lambrecht et al. (1982a) reported the incidence of hepatoma at the 200 ppm level to be 7.7% in males and 6.7% in females; at the 400 ppm level the incidence was 5% in females and 14.3% in males. These figures are based on the numbers of animals at risk at the time of the earliest observed tumor. The time to first tumor was relatively late in the study, 276 days for males and 255 days for females of the lower dose and 153 days for males and 299 days for females at the higher dose. Since the test animals were systematically sacrificed from 3 months onward, the time to tumor figures should be reasonably close to actual time to tumor. Table 12-19 shows the results reported by Lambrecht et al. (1982a).

The tumorigenicity and carcinogenicity of hexachlorobenzene has been demonstrated by one lifetime study in hamsters. Additional suggestive evidence for tumorigenicity is found in a 90-day study in another laboratory. In both cases hepatomas resulted. The longer period of exposure also produced thyroid adenomas and metastatic liver haemangioendothelioma.

12.3.5.2. MOUSE STUDIES --

12.3.5.2.1. **Cabral et al. (1979)** -- Cabral et al. (1979) reported that outbred Swiss mice were fed hexachlorobenzene (99.5% purity) in their diets for up to 120 weeks. The hexachlorobenzene content of the diet was monitored periodically during the study and the diet was found to be free of aflatoxins. The exposure levels used were 50, 100 and 200 ppm corresponding to dosages of 6, 12 or 24 mg/kg/day based on body weight and food intake averages. One other test group was given 300 ppm (36 mg/kg/day) for only 15 weeks and retained on an hexachlorobenzene-free diet for the remainder of the study.

TABLE 12-19

Effect of HCB on Hamsters: Liver Tumors and Other Liver Lesions[a]

Sex	HCB (ppm)	PC+C[b] Incidence	BDH[c] Incidence	Day First Observed	Hepatomas Incidence	Day First Observed
M	0	3/50	0		0	
	200	48/49	0		1/13	276
	400	50/50	1/25	101	1/20	153
F	0	10/43	0		0	
	200	48/49	1/6	340	1/15	255
	400	45/45	2/20	174	1/7	299

[a] Source: Lambrecht et al., 1982a

[b] Precirrhotic + cirrhotic

[c] Biliary duct hyperplasia

HCB = Hexachlorobenzene

Growth rates were monitored but not given in detail in the published report. The investigators stated that among female mice there was a reduced growth rate for all doses except in the 12 mg/kg/day dose group and among males for all doses except in the 6 mg/kg/day dosage group.

Survival times were reported in detail. Survival was essentially unaffected in the two lower dosage level groups at 50 weeks, while at that time it was down by 60% of the original number in the females and 52% of the original number in the males in the highest dosage group. By 70 weeks on test the survival was decreased in the two lower dose groups as well, and in the highest dose group it was down to 14% in females and 10% in males. At 90 weeks there were only four surviving males out of the 50 and no surviving females in the highest dosage group as compared with 96 and 100% survival in the female and male controls.

The yield of tumors in this study is given in Tables 12-20 and 12-21. In Table 12-20, the effective number of animals is the number of animals alive at the earliest time a liver cell tumor was observed in each group while in Table 12-21 the effective number of animals is that number of animals alive at the earliest appearing tumor for any site in the body within that group. There was a statistically significant elevation in the incidence of liver cell tumors at the high dose in females and a marginal increase in high-dose males, with a positive dose-related trend in both cases. There was also a dose-dependent decrease in latent period and a dose-dependent increase in the size and multiplicity of liver cell tumors (Table 12-20). The liver cell tumors were subsequently defined as hepatomas (Cabral, 1983).

TABLE 12-20

Liver Tumor Incidence in Mice Fed HCB[a]

Group	Effective[b] No. Animals	Mice with LCT		Node Size (mm)		Multiplicity		Age at Death (weeks)	
		No.	%	<8	≥8	Single	Multiple	Range	Average
HCB 100	F 12	3	25	2	1	1	2	87-104	98
	M 12	3	25	1	2	2	1	83-98	89
HCB 200	F 26	14	54	5	9	3	11	47-85	67
	M 29	7	24	4	3	2	5	46-101	73
HCB 300 (15 weeks exposure)	F 10	1	10	--	1	1	--	101	101
	M 3	1	33	--	1	--	1	97	97

[a] Source: Cabral et al., 1979

[b] Survivors at time first LCT was observed in each group

LCT = Liver cell tumors

HCB = Hexachlorobenzene

TABLE 12-21

Tumor Data on Mice Fed HCB[a]

Group	Initial No. Animals	Effective[b] No. Animals	Animals with Tumors													
			TBA[c]		Lymphomas			Lung			Liver-cell		Gonads		Other	
			No.	%	No.	%	Average Age at Death (weeks)	No.	%	Average Age at Death (weeks)	No.	%	No.	%	No.	%
Control	F 50	49	39	80	21	43	89.6	14	29	89.0	0	0	3	6	9[d]	18
	M 50	47	22	47	12	26	80.8	13	28	83.8	0	0	0	0	4[e]	9
HCB 50	F 30	30	21	70	16	53	69.8	4	13	84.5	0	0	2	7	2[f]	7
	M 30	30	15	50	13	43	73.7	4	13	87.0	0	0	0	0	0	0
HCB 100	F 30	30	13	43	5	17	94.4	6	20	83.5	3	10	1	3	3[g]	10
	M 30	29	10	34	7	24	70.4	0	0	--	3	10	0	0	1[h]	3
HCB 200	F 50	41	19	46	5	12	58.2	2	5	66.5	14	34	1	2	1[i]	2
	M 50	44	12	27	4	9	53.2	4	9	82.5	7	16	1	2	0	0
HCB 300 (15 weeks)	F 30	26	20	77	8	31	97.7	4	15	91.2	1	4	3	12	8[j]	31
	M 30	16	5	31	3	19	68.6	2	13	83.5	1	6	0	0	0	0

[a] Source: Cabral et al., 1979

[b] Number of survivors at moment of appearance of first tumor at any site in each group

[c] In relation to the effective number

[d] Skin fibrosarcoma, uterine haemangioendothelioma, one skin haemangioendothelioma, two adrenal adenoma, two mammary adenoma

[e] Urinary bladder transition cell carcinoma, one liver haemangioendothelioma, one skin haemangioendothelioma, one skin fibrosarcoma

[f] One uterine haemangioendothelioma, one skin fibrosarcoma

[g] Two skin fibrosarcoma, one skin haemangioendothelioma

[h] One skin squamous-cell carcinoma

[i] One intestinal leiomyosarcoma

[j] One skin fibrosarcoma, two liver haemangioendothelioma, one cecum carcinoma, one stomach papilloma, one skin haemangioendothelioma, one uterine adenoma, one mammary adenoma

HCB = Hexachlorobenzene

In this study there was a high incidence of both lymphoma and lung tumors in control mice. A dose-related decrease in the incidence of lymphomas appears in the treated groups. The investigators attributed this to the decreased survival time of hexachlorobenzene-treated animals. This seems reasonable but does not explain the reduction in lung tumors in the 50 ppm (6 mg/kg/day) group when they are compared to controls, since there was not an appreciable reduction of lifespan in this low dose group.

This study by Cabral (1979) demonstrates the tumorigenicity of hexachlorobenzene in Swiss mice by the significant increase in liver cell tumors in both sexes and by the demonstration of dose-dependency in the response with respect to tumor incidence, tumor size, multiplicity and latent period duration. Tumorigenicity was detected as low as 12 mg/kg bw/day (100 ppm) for lifetime exposure but not at 6 mg/kg bw/day (50 ppm).

12.3.5.2.2. Lambrecht et al. (1982b) -- Swiss mice exposed to hexachlorobenzene for only 90 days at levels of 100 and 200 ppm in the diet showed degenerative changes of liver and kidneys when examined at various intervals after they were removed from the hexachlorobenzene-containing diet (Lambrecht et al., 1982b). Although liver tumors were not reported, treated animals showed lymphosarcomas in both dosage groups in both sexes at levels significantly above those of controls. Exposure to hexachlorobenzene in this instance produced leukemogenic changes. The animals were not permitted to live beyond selected intermediate sacrifice dates, so it was not possible to determine whether survivors would have developed liver or other tumors. The method of preparation of the hexachlorobenzene-containing diet may have been different in the Cabral et al. (1979) and Lambrecht et al. (1982b) studies, but detailed information was not presented in the Lambrecht et al. (1982b) abstract.

Mice may be somewhat less sensitive than hamsters to hexachlorobenzene as evidenced by the difference in incidence of hepatoma formation at various doses. These animal species may differ in the distribution of the hexachlorobenzene into various tissue compartments (Lambrecht et al., 1981), and differ in rates of metabolism and absorption. Administration of the same levels of hexachlorobenzene in the feed can be expected to give different effective dosages.

12.3.5.2.3. Shirai et al. (1978) -- Shirai et al. (1978) administered hexachlorobenzene to male ICR mice (35 animals/group) at levels of 10 or 50 ppm in the diet for periods of 24 weeks. Polychlorinated terphenyl was given alone to another group at 250 ppm, and in combination with 50 ppm hexachlorobenzene to a third group. Animals were examined histologically at 40 weeks.

Final body weights were slightly lower in the hexachlorobenzene-treated groups while liver weights were higher. Examination of the livers showed that the hexachlorobenzene-treated groups had hypertrophy of the centrilobular area at both doses. No liver tumors were found in either group. The total intake of hexachlorobenzene was calculated to be 8.4 and 35.3 mg/mouse of the 10 ppm and 50 ppm groups, respectively.

Polychlorinated terphenyl alone, at 250 ppm (total dose 207.4 mg/mouse) gave 3/28 (10.7%) nodular hyperplasia. When this same level of polychlorinated terphenyl was given along with hexachlorobenzene at 50 ppm (total dose 36.9 mg/ mouse) there were 23/26 (88.5%) nodular hyperplasia and 8/26 (30.8%) hepatocellular carcinoma. This response indicates that hexachlorobenzene can enhance the carcinogenic potency of polychlorinated terphenyl.

The duration of administration, 24 weeks, in this mouse study and the doses used were below those used in the Cabral (1979) study on Swiss mice and also below the levels used in the 13-week study by Lambrecht (1982b) on Swiss mice. Therefore, it is not surprising that hepatomas were not found when hexachlorobenzene was given alone. The occurrence of liver lesions, however, does indicate target organ toxicity.

These three studies in mice demonstrate the tumorigenicity of hexachlorobenzene with respect to the induction of hepatomas, the leukemogenic effect of subchronic exposure and the ability of hexachlorobenzene to enhance the carcinogenic effect of another compound.

12.3.5.3. RAT STUDIES --

12.3.5.3.1. **Smith and Cabral (1980)** -- The carcinogenic potential of hexachlorobenzene was tested in several different laboratories in rats. In one study (Smith and Cabral, 1980) small numbers of female Agus rats, and even smaller numbers of female Wistar rats, were used. There were 12 control and 14 treated Agus rats and 4 control and 6 treated Wistar rats. The hexachlorobenzene was analytical grade (99.5% purity) dissolved in arachis oil and mixed with the feed to give 100 ppm in the diet. This dietary level supplied an average daily dose of 6-8 mg/kg/day to the rats.

In this study the Agus rats showed signs of porphyria after 3 months exposure to hexachlorobenzene, but other toxic manifestations were not found. The investigators stated that "there was a steady decline in body weight to eventually 80% of control animals" (Table 12-22). Examination of the weight data presented in the publication indicates that this interpretation is based upon comparison of "final" average weight in control (286±19 g) and treated (225±16 g) animals (Table 12-22), representing a 21% difference in weight. This method of comparison can be misleading since the final weights represent accumulated differences in growth rates and varying

TABLE 12-22

Body Weights of Female Agus Rats Fed Hexachlorobenzene for 90 Weeks[a]

Weeks of Diet	Body Weight (g)		% Difference
	Control	HCB	
0	46 ± 6 (8)	45 ± 24 (9)	2
10	191 ± 5	180 ± 17	6
30	236 ± 13	212 ± 13[b]	10
50	257 ± 17	221 ± 19[c]	14
90	286 ± 19 (8)	225 ± 16 (7)[c]	21

[a] Source: Smith and Cabral, 1980

[b] Significantly different from controls as assessed by Student's t-test $p<0.01$

[c] $p<0.001$

Female Agus rats were fed HCB (100 ppm) in MRC 41B diet for 90 weeks and then killed. Weights are menas (no. of animals in parentheses) ± S.D.

HCB = Hexachlorobenzene

composition of the groups because of animal deaths. An effect produced, even transiently, at an early age, may persist in the figures, even though all subsequent growth may be normal. Growth rates, rather than absolute difference in weights provide a more suitable picture of the animal response. Growth rates for the time intervals reported were calculated based on the data given in the publication and are shown in Table 12-23. The equation used was:

$$R = \frac{\text{weight at end of interval} - \text{weight at start of interval}}{\text{weight at start of interval}} \times 100$$

According to this calculation both groups of animals grew during each time interval.

The survival of the treated Agus rats was good; one test animal was sacrificed at 52 weeks and a second one died of pneumonia at 70 weeks. Both of these animals had liver cell tumors found by histologic examination. Another five treated animals were sacrificed at 75 weeks and the remaining seven treated animals lived until the end of the experiment at 90 weeks. Among controls, one was killed at 63 weeks and three more at 75 weeks. The remaining eight were killed at 90 weeks.

No control animals had liver pathology. In contrast, 14/14 (100%) of the treated Agus rats had liver tumors; the earliest of these was detected at 52 weeks. The livers of the treated animals were grossly enlarged and some of the tumors were 1.5-2 cm in diameter. Although one liver cell tumor was described as pedunculated, histopathology detail was not given, except to note the absence of metastases in all cases. Four of the six (67%) Wistar rats also had liver cell tumors and none of the four controls showed such pathology at 75 weeks.

TABLE 12-23

Growth Rates for Female Agus Rats on a Diet Containing 100 ppm HCB*

Interval (on diet)	Average Growth Rate %/week	
	Control	Treated
0-10 weeks	31.5	30.0
10-30 weeks	1.2	0.89
30-50 weeks	0.45	0.22
50-90 weeks	0.28	0.05

*Source: Calculated from Smith and Cabral, 1980

HCB = Hexachlorobenzene

In this rat study hexachlorobenzene was a potent inducer of liver tumors, causing a 100% incidence with the earliest tumor observed at 52 weeks. It is important to determine whether the magnitude of the effect is all attributable to the hexachlorobenzene or whether contaminants, unusual characteristics of the test animals, or procedural factors were operative in this study. In this context the following points are noted.

First, historical control data on tumor incidence for Agus rats were not available, but, according to Cabral (1983), the Agus rat is a strain particularly sensitive to porphyria and hepatic tumors. In regard to the question of contaminants, peanut oil is generally believed to be free of aflatoxins [they are destroyed in processing (NAS, 1977)] and the feed was analyzed for both aflatoxins and dibenzofurans and found to be free of both (Cabral, 1983). Absorption is another factor to consider. The absorption of the hexachlorobenzene in these animals might be enhanced by dissolution in the arachis oil.

12.3.5.3.2. **Lambrecht et al. (1983a,b, 1984)** -- Another study on rats was carried out by Lambrecht et al. (1983a,b, 1984). In this study 94 Sprague-Dawley rats of each sex for each dosage and control groups were used. Four animals of each group were sacrificed at each of 10 intervals: 0, 1, 2, 3, 4, 16, 32, 48 and 64 weeks. The remaining 54 animals of each group were allowed to continue until they died, or to the end of the 2 years. The number of animals at risk was considered to be those that survived at least 12 months, since this was the earliest time to tumor. This number would be, at minimum, 54 plus some animals from the last sacrifice time.

The hexachlorobenzene was highly purified and the prepared diet monitored for hexachlorobenzene levels periodically. The preparation was also analyzed for aflatoxins and found to be negative. The test diet was prepared by mixing the hexachlorobenzene with dextrose and Wayne laboratory feed (1.5 g hexachlorobenzene + 98.5 g dextrose + 9.9 kg lab chow to give 150 ppm hexachlorobenzene). Half the amount of hexachlorobenzene was used in the mix for the 75 ppm hexachlorobenzene level. This oil-free vehicle is different from the vehicle used by both Smith and Cabral (1980) and Arnold et al. (1983). The hexachlorobenzene was well absorbed as shown by progressive accumulation in fat which was measured in this study.

Based on an average food consumption of 22.6 g/rat/day for males and 16.5 g/rat/day for females, and on an average adult weight for females of 265 g and for males of 400 g, the low dose was calculated to be 4-5 mg/kg/day and the high dose, 8-9.5 mg/kg/day. In order to compare the results obtained in this study with those obtained in Sprague-Dawley rats by Arnold et al. (1983), more detailed calculation of doses at different time periods on test are given in Table 12-24.

The administration of hexachlorobenzene in the diet at these doses in the Lambrecht et al. (1983a) chronic feeding study in rats resulted in liver pathology just before the appearance of hepatoma or hepatocellular carcinoma. Pathology observed at the early sacrifice time included parenchymal degeneration, preneoplastic foci and adenoma. At 48 and 64 weeks of the test females had gross liver tumors which measured between 1 and 2 mm^2. Porphyria was also detected.

Rats that lived 12 months or longer showed a significant increase in hepatoma incidence in both sexes. A statistically significant increase in the incidence of hepatocellular carcinoma was found at both doses in the females, and in males a slight non-significant increase was found. None of the liver cell tumors metastasized. Table 12-25 summarizes the findings.

TABLE 12-24

Dosage Levels in the Chronic Feeding Study of Hexachlorobenzene in Sprague-Dawley Rats[a] (mg/kg/day)

Time on Diet[b] (weeks)	Males		Females	
	75 ppm	150 ppm	75 ppm	150 ppm
0	19.5	37.0	16.1	32.2
26	3.2	7.1	3.7	8.7
52[c]	3.3	6.4	3.8	8.0
79	3.4	6.7	3.5	8.4
99	6.2	10.0	4.3	10.6

[a] Source: Calculations and data provided by Lambrecht, 1984

[b] The animals were 3 weeks old when placed on test

[c] At 52 weeks on test the males consumed an average of 24.7 g of the diet/day and weighed an average of 553.7 g. The females consumed an average of 16.0 g diet/day and weighed an average of 311.7 g.

TABLE 12-25

Liver and Kidney Tumors in Sprague-Dawley Rats Given Hexachlorobenzene in the Diet for up to 2 years[a,b]

Exposure Level	Hepatoma		Hepatocellular Carcinoma		Renal Cell Adenoma		Renal Cell Carcinoma	
	M	F	M	F	M	F	M	F
0	0/54	0/52	0/54	0/52	7/54	1/52	0/54	1/52
percentage	0	0	0	0	13	2	0	2
75 ppm	10/52	26/56	3/52	36/56	41/52	7/56	0/52	2/56
percentage	19	46	6	64	79	13	0	4
150 ppm	11/56	35/55	4/56	48/55	42/56	15/54	0/56	2/54
percentage	20	64	7	87	75	28	0	4

[a] Source: Lambrecht et al., 1983a,b; Lambrecht, 1983

[b] The diet was prepared without solubilization of the hexachlorobenzene, but by mixing it as a pulverized solid.

Renal cell adenoma was found to be significantly elevated in both sexes but with greater frequency in males. In this study the control male group had a high incidence of renal cell adenoma which was not explained; nevertheless, the increase in the hexachlorobenzene-treated animals was statistically significant. The incidence of renal cell carcinoma in treated animals was not significantly increased over control animals in either males or females.

In an updated report from this laboratory (Peters et al., 1983) histopathology details were supplied. These data show that in addition to the liver and kidney lesions there was an increase in adrenal pheochromocytoma in female rats which was statistically significant at both 75 and 150 ppm. Females also had elevated incidences of adrenal cortical adenoma and hemangioma in the treated groups. Among males the background incidence of adrenal pheochromocytomas is high (76.5%), making it difficult to determine whether the 90.6% incidence found in the 150 ppm group has any biological significance. Other adrenal neoplastic and non-neoplastic lesions were detailed: hyperemia and/or congestion, cortical hyperplasia, preneoplastic foci, cysts, lipoma and adenocarcinoma; none of these were elevated in the treated animals. The adrenal tumor incidences are given in Table 12-26.

One point to consider in the interpretation of the results, particularly in terms of their application to risk assessment, is the form in which the hexachlorobenzene was administered in the diet. The absorption from a particulate form introduces an additional possible exposure route, namely, from the food preparation by inhalation. This consideration does not invalidate the study, but raises the question of the actual exposure levels if an additional route of exposure was occurring in the same experiment simultaneously with oral ingestion.

TABLE 12-26

Adrenal Tumors in Sprague-Dawley Rats Given Hexachlorobenzene in the Diet for up to 2 Years[a,b]

MALES

Days on diet	400-599			600+		
Exposure ppm hexachlorobenzene	0	75	150	0	75	150
Number of tissues examined	17	23	28	34	25	23
Cortical adenoma (%)	3	2	6	6	3	4
Pheochromocytoma (%)	3 (17.6)	6 (26.1)	9 (32.1)	26 (76.5)	17 (68)	21 (91.3)
Hemangioma (%)	0	0	0	0	0	0

FEMALES

Days on diet	400-599			600+		
Exposure ppm hexachlorobenzene	0	75	150	0	75	150
Number of tissues examined	12	5	13	35	47	32
Cortical adenoma (%)	0	3	2	2 (5.7)	11 (23.4)	6 (18.8)
Pheochromocytoma (%)	0	0	2	5 (14.3)	31 (66)	29 (90.6)
Hemangioma (%)	0	0	2	3 (8.5)	8 (17)	5 (15.6)

[a] Source: Peters et al., 1983

[b] The diet was prepared without solubilization of the hexachlorobenzene, but by mixing it as a pulverized solid.

12.3.5.3.3. **Arnold et al. (1983)** -- Another study (an unpublished 1983 draft, amended in 1984) on Sprague-Dawley rats was carried out at the Canadian Health Protection Branch of the Canadian Health Ministry (Arnold et al., 1983). This 2-generation study has been completed, but not yet published. Our data were derived from the draft of the manuscript to be submitted for publication and from personal communication with Dr. Arnold of the Canadian Health Ministry.

In this study hexachlorobenzene (99% pure) was administered to parental male and female Sprague-Dawley rats for 3 months. These animals were mated at that time and the females continued to receive hexachlorobenzene-containing diets during pregnancy and throughout lactation. At weaning, 50 pups of each sex were separated and fed for the remainder of their lifetime on hexachlorobenzene-containing diets. Controls were fed diets free of hexachlorobenzene. The range of doses used in this study is considerably lower than those used by either Smith and Cabral (1980) or Lambrecht et al. (1983a,b). Table 12-27 shows the doses used in the Arnold et al. study at particular points in time since the doses were not adjusted throughout the study. These doses represent a greater exposure to the test animals from the point of view of exposure duration, since the F_1 animals were exposed in utero and during nursing in addition to their exposure from feeding on an hexachlorobenzene-containing diet. Total doses cannot be calculated since the actual dose received during nursing is not known.

Arnold et al. (1983) found no differences in treated animals when compared to controls with respect to growth rates, food consumption, hematology or survival except at the highest dose used. At this dose, F_1 pups had a significantly increased mortality.

TABLE 12-27

Exposure Levels in the Chronic Feeding, 2-Generation Study of Hexachlorobenzene in Sprague-Dawley Rats[a] (mg/kg/day)

Time on Diet[b] (weeks)	Exposure Level			
	0.32 ppm	1.6 ppm	8.0 ppm	40.0 ppm
MALES				
1	0.04	--	0.93	4.85
30[c]	0.01	0.06	0.29	1.5
70	0.01	0.05	0.25	1.3
FEMALES				
1	0.04	0.17	0.84	4.64
30[c]	0.02	0.08	0.40	1.9
70	0.01	0.06	0.32	1.6

[a] Source: Calculations and data provided by Arnold, 1984

[b] The animals were placed on feed at 6 weeks of age.

[c] The mean body weight of male controls was 663 g and for the highest dose group males 653 g. The mean weekly food consumption for male controls at that time was 178 g and for the highest dose group 169 g. Females of the same age weighed 351 g for controls and 353 g for the highest dose treated group and the mean weekly food consumption was 113 and 118 g, respectively.

Histopathology showed that F_1 females had a significant elevation in neoplastic liver nodules and in adrenal pheochromocytoma in the high dose females compared to controls (Table 12-28). There was also a significant positive dose-related trend in the incidence of these tumors in F_1 females.

Among F_1 males, in the highest dose group parathyroid tumors were significantly increased: 25% (12/48) in the treated groups and 4.2% (2/48) among controls. Females also showed a few parathyroid tumors in the two highest dose groups and none in controls or in the two lowest dose groups, but the differences were not statistically significant. Table 12-28 gives the tumor incidences. Although kidney tumors were not reported to be elevated, there was an increased chronic nephrosis in the F_1 treated animals.

12.3.5.3.4. Arnold et al. (1983) -- In another study by Arnold et al. (1983) which was related to the 2-generation study, the effect of vitamin A in a synthetic diet was tested in conjunction with hexachlorobenzene. This was a 1-generation study and the level of hexachlorobenzene was the same as the highest dose of the 2-generation study, 40 ppm. There were six separate groups of 50 animals each and the experiment ran for 119 weeks. At 29 weeks and at 49 weeks five animals from each group were sacrificed and evaluated histologically. The six groups are shown in Table 12-29. The vitamin A did not apparently alter the effects of hexachlorobenzene. The number of animals with parathyroid tumors and adrenal pheochromocytomas was somewhat elevated in all the cases in which hexachlorobenzene was administered compared with the total cases with the three levels of vitamin A and no hexachlorobenzene. The significance of these tumor incidences cannot be determined by simple comparison because it was also found in the study that vitamin A had an effect on the background level of some common tumors and these data have not yet been completely analyzed.

TABLE 12-28

Tumors in Organs that Showed Statistical Differences from Control in F_1 Sprague-Dawley Rats Treated with Hexachlorobenzene[a] [incidence (%)]

Dose at 30 weeks (mg/kg bw/day)	Parathyroid Adenoma		Adrenal Pheochromocytoma		Hepatocellular Carcinoma		Neoplastic Liver Nodules	
	Males	Females	Males	Females	Males	Females	Males	Females
Controls	2/48 (4.2)	0/49 (0)	10/48 (20.8)	2/49 (4.1)	0/48 (0)	0/49 (0)	2/48 (4.2)	0/49 (0)
0.01-0.02	4/48 (8.3)	0/49 (0)	12/48 (25.0)	4/49 (8.0)	2/48 (4.2)	0/49 (0)	0/48 (0)	0/49 (0)
0.06-0.08	2/48 (4.2)	0/50 (0)	7/48 (14.6)	4/50 (8.0)	1/48 (2.1)	0/49 (0)[b] 1/49 (2.0)[b]	0/48 (0)	2/50 (4.0)
0.29-0.40	1/49 (2.0)	1/49 (2.0)	13/49 (26.5)	4/49 (10.2)	3/49 (6.1)	0/50 (0)	2/49 (4.1)[b] 3/49 (6.1)[b]	2/49 (4.1)[b] 3/49 (6.1)[b]
1.5-1.9	12/49 (24.5)	2/49 (4.1)	17/49 (34.7)	17/49 (34.7)	0/49 (0)	0/49 (0)[b] 1/49 (2.0)[b]	1/49 (2.0)	10/49 (20.4)[b] 9/49 (18.4)[b]
Other statistical tests								
IARC trend test	$p \leq 0.01$	$p \leq 0.01$		$p \leq 0.01$				$p \leq 0.01$
Armitage time-related trend test	$p \leq 0.01$	$p \leq 0.05$		$p \leq 0.01$				$p \leq 0.01$
Fisher exact treated vs. control				$p \leq 0.01$				$p \leq 0.01$

[a] Source: Arnold et al., 1983; Arnold, 1984

[b] Different results of two different pathologists reading the same slides

TABLE 12-29

Parathyroid and Adrenal Pheochromocytomas in Sprague-Dawley Rats Maintained on Synthetic Diets of Varying Vitamin A Content and With or Without Hexachlorobenzene*

Group	No. with Parathyroid Tumors	No. with Adrenal Pheochromocytoma
Controls on diet with normal vitamin A content	3	3
Control diet + 40 ppm HCB	4	6
Diet with 0.1 times normal vitamin A	0	2
Diet with 0.1 times normal vitamin A + 40 ppm HCB	0	2
Diet with 10X vitamin A	1	4
Diet with 10X vitamin A + 40 ppm HCB	3	7
Total without HCB	4	9
Total with HCB 40 ppm	7	15

*Source: Arnold et al., 1983

HCB = Hexachlorobenzene

12.3.5.4. DISCUSSION OF RAT STUDIES -- It seems appropriate to compare the findings of Smith and Cabral (1980) in Agus and Wistar rats, Lambrecht et al. (1983a,b) and Arnold et al. (1983) in Sprague-Dawley rats. None of the three studies agree precisely on all four of the tumor target organs: Smith and Cabral reported liver tumors, Lambrecht reported liver, adrenal and kidney tumors and had some liver carcinomas not found by Smith and Cabral. Arnold found adrenal and parathyroid tumors and neoplastic liver nodules but no increase in kidney tumors. We find that, although differences do occur, the results are not contradictory for the following reasons:

1. The dosages used in the Arnold et al. (1983) study were below those used by either Smith and Cabral (1980) or Lambrecht et al. (1983a,b). The range of doses used by Smith and Cabral was given as 6-8 mg/kg/day and those used by Lambrecht were 3-9 mg/kg bw/day. Those of Arnold were, at most, between 1.5 and 2.0 mg/kg bw/day.
2. There were notable differences in the animals used: in the case of Smith and Cabral the liver tumor susceptible strain of Agus rat was used, although tumors were also found with Wistar rats. We do not have full data on historical tumor incidences in these animals to allow for more detailed evaluation.
3. The conditions of the Smith and Cabral study and those of Lambrecht were both different from the 2-generation study of Arnold. The possible effects prenatal exposure could have on metabolism of xenobiotics renders this an important consideration.
4. The method of preparation of the hexachlorobenzene in the diet was different in that both Smith and Cabral and Arnold used arachis oil and corn oil as hexachlorobenzene solvents while Lambrecht did not use an oil vehicle. Absorption characteristics are known to depend upon the vehicles used.

5. The Sprague-Dawley animals used by Arnold may have more fat than those used by Lambrecht as they were somewhat larger. Distribution into different tissue compartments, especially into fat where it is likely the hexachlorobenzene is at least temporarily stored, is likely to alter the effective concentration in target tissues. In this regard the hexachlorobenzene is known to concentrate in adrenal tissue; the degree of such concentration may well vary with strain or diet of the host animals.

In summary, orally administered hexachlorobenzene has induced hepatocellular carcinoma in male Sprague-Dawley (S-D) rats as well as hepatomas in female Agus and Wistar rats and in S-D rats of both sexes. At the lowest dose used in any of the studies (40 ppm in the diet or 1.5 mg/kg/day), neoplastic nodules were induced in S-D rats, whereas hepatocellular carcinomas occurred in the same strain at a higher dose (4-5 mg/kg/day). Adrenal pheochromocytoma was significantly elevated in two separate studies in female S-D rats. In the same strain one investigator reported parathyroid tumors and a different investigator reported kidney tumors; neither of these findings has been repeated by other authors. Table 12-30 summarizes this information.

12.3.5.5. OTHER STUDIES -- In addition to the studies described on hamsters, mice and rats there are a few studies which cover specific kinds of tests other than lifetime exposure and examination of all potential target tissues for tumorigenic or carcinogenic response.

One such study was that of Theiss et al. (1977) in which the experiment was designed to detect only pulmonary tumors following i.p. injection of organic chemicals found as contaminants of drinking water. In this assay hexachlorobenzene was one of the chemicals tested. Strain A mice were given three dosage levels of hexachlorobenzene with the top level as the MTD. A total of 24 injections over a period of 8 weeks were given to 20 mice/group.

TABLE 12-30

Qualitative Comparison of Tumor Development in Rats Following Hexachlorobenzene Administration in Different Studies

Strain/Sex	Dosage (lowest dose that produced tumor)	Liver	Kidney	Adrenal	Parathyroid	Reference
Agus/Female	100 ppm (6-8 mg/kg bw/day)	liver-cell tumor (F)	NA	NA	NA	Smith and Cabral, 1980
Wistar/Female	prepared by dissolving in oil and mixing oil with food	liver-cell tumor (F)	NA	NA	NA	Smith and Cabral, 1980
Sprague-Dawley/ Male and female	75 ppm (3-4 mg/kg bw/day) prepared in feed sans oil vehicle	hepatocellular carcinoma (M&F) hepatoma (M&F)	renal cell adenoma (M&F)	pheochromo-cytoma (F) cortical adenoma (F)	NA	Lambrecht, 1983a,b
Sprague-Dawley/ Male and female F_1 animals of 2-generation study	40 ppm (0.3-1.5 mg/kg bw/day) prepared in oil and mixing oil with food at weaning -- animals exposed in utero and during nursing	neoplastic liver nodules (F)	not found	pheochromo-cytoma (F)	adenoma (M)	Arnold, 1983

NA = It is not known whether or not these tissues were examined.

The total doses received were 190, 480 and 960 mg/kg. Lungs were the only organ examined and hexachlorobenzene did not increase tumor incidence in that organ. The study ran for 32 weeks. Although this assay has proved useful in detecting some pulmonary carcinogens, it is not designed to detect other tumors.

In another study on beagle dogs in which hexachlorobenzene was given in daily gelatin capsules to 30 animals of each sex/dosage group the duration of the study was only 1 year. Although this is not a long enough period of time for a carcinogenicity study in dogs, it is of interest to note that the doses of 100, 10, 1 and 0.1 mg/kg bw/day produced a number of toxic manifestations in the liver including bile duct hyperplasia, hepatomegaly and liver necrosis. This study is more appropriately considered under chronic toxicity.

Finally, Pereira et al. (1982) designed a study to determine whether hexachlorobenzene increased γ-glutamyltranspeptidase-positive foci in rats. These foci are believed to be preneoplastic in the liver. The assay is designed to test initiation/promotion in this case by employing diethyl-N-nitrosamine (DENA) as the initiating agent and hexachlorobenzene as the promoter. Unfortunately, there are some errors in reporting of the results in the published paper and some important controls were not included (Pereira, 1983). We have not yet received a corrected manuscript.

12.3.5.6. **QUANTITATIVE ESTIMATION** -- Among the six chlorinated benzenes reviewed in this document, only hexachlorobenzene provides sufficient data for a risk estimate. This quantitative section deals with estimation of the unit risk for hexachlorobenzene as a potential carcinogen in air and water, and with the potency of hexachlorobenzene relative to other carcinogens that have been evaluated by the U.S. EPA Carcinogen Assessment Group

(CAG). The unit risk for an air or water pollutant is defined as the lifetime cancer risk to humans from daily exposure to a concentration of 1 $\mu g/m^3$ of the pollutant in air by inhalation, or to a concentration of 1 $\mu g/\ell$ in water by ingestion.

The unit risk estimate for hexachlorobenzene represents an extrapolation below the dose range of experimental data. There is currently no solid scientific basis for any mathematical extrapolation model that relates exposure to cancer risk at the extremely low concentrations, including the unit concentration given above, that must be dealt with in evaluating environmental hazards. For practical reasons the correspondingly low levels of risk cannot be measured directly either by animal experiments or by epidemiologic study. Low dose extrapolation must, therefore, be based on current understanding of the mechanisms of carcinogenesis. At the present time the dominant view of the carcinogenic process involves the concept that most cancer-causing agents also cause irreversible damage to DNA. This position is based in part on the fact that a very large proportion of agents that cause cancer are also mutagenic. There is reason to expect that the quantal response that is characteristic of mutagenesis is associated with a linear (at low doses) non-threshold dose-response relationship. Indeed, there is substantial evidence from mutagenicity studies with both ionizing radiation and a wide variety of chemicals that this type of dose-response model is the appropriate one to use. This is particularly true at the lower end of the dose-response curve; at high doses there can be an upward curvature, probably reflecting the effects of multistage processes on the mutagenic response. The linear non-threshold dose-response relationship is also consistent with the relatively few epidemiologic studies of cancer responses to specific agents that contain enough information to make the evaluation possible (e.g., radiation-induced leukemia, breast and thyroid cancer, skin cancer induced by arsenic in drinking water, liver cancer induced by aflatoxins in the diet). Some supporting evidence also exists from animal experiments (e.g., the initiation stage of the two-stage carcinogenesis model in rat liver and mouse skin).

Because its scientific basis, although limited, is the best of any of the current mathematical extrapolation models, the non-threshold model, which is linear at low doses, has been adopted as the primary basis for risk extrapolation to low levels of the dose-response relationship. The risk estimates made with such a model should be regarded as conservative, representing the most plausible upper limit for the risk (i.e., the true risk is not likely to be higher than the estimate, but it could be lower).

For several reasons, the unit risk estimate based on animal bioassays is only an approximate indication of the absolute risk in populations exposed to known carcinogen concentrations. First, there are important species differences in uptake, metabolism and organ distribution of carcinogens, as well as species differences in target site susceptibility, immunological responses, hormone function, dietary factors and disease. Second, the concept of equivalent doses for humans compared to animals on a mg/surface area basis is virtually without experimental verification as regards carcinogenic response. Finally, human populations are variable with respect to genetic constitution and diet, living environment, activity patterns and other cultural factors.

The unit risk estimate can give a rough indication of the relative potency of a given agent as compared with other carcinogens. Such estimates are, of course, more reliable when the comparisons are based on studies in which the test species, strain, sex and routes of exposure are similar.

The quantitative aspect of carcinogen risk assessment is addressed here because of its possible value in the regulatory decision-making process, e.g., in setting regulatory priorities, evaluating the adequacy of technology-based controls, etc. However, the imprecision of presently available technology for estimating cancer risks to humans at low levels of exposure should be recognized. At best, the linear extrapolation model used here provides a rough but plausible estimate of the upper limit of risk -- that is, with this model it is not likely that the true risk would be much more than the estimated risk, but it could be considerably lower. The risk estimates presented in subsequent sections should not be regarded, therefore, as accurate representations of the true cancer risks even when the exposures involved are accurately defined. The estimates presented may, however, be factored into regulatory decisions to the extent that the concept of upper-risk limits is found to be useful.

12.3.5.6.1. Procedures for the Determination of Unit Risk --

12.3.5.6.1.1. Low Dose Extrapolation Model. The mathematical formulation chosen to describe the linear non-threshold dose-response relationship at low doses is the linearized multistage model. This model employs enough arbitrary constants to be able to fit almost any monotonically increasing dose-response data, and it incorporates a procedure for estimating the largest possible linear slope (in the 95% confidence limit sense) at low extrapolated doses that is consistent with the data at all dose levels of the experiment.

Let P(d) represent the lifetime risk (probability) of cancer at dose d. The multistage model has the form

$$P(d) = 1 - \exp\left[-(q_0 + q_1 d + q_1 d^2 + \ldots + q_k d^k)\right]$$

where

$$q_i \geq 0, \; i = 0, 1, 2, \ldots, k$$

Equivalently,

$$P_t(d) = 1 - \exp [-(q_1d + q_2d + \ldots q_kd^k)]$$

where

$$P_t(d) = \frac{P(d) - P(0)}{1 - P(0)}$$

is the extra risk over background rate at dose d.

The point estimate of the coefficients q_i, $i = 0, 1, 2, \ldots, k$, and consequently, the extra risk function, $P_t(d)$, at any given dose d, is calculated by maximizing the likelihood function of the data.

The point estimate and the 95% upper confidence limit of the extra risk, $P_t(d)$, are calculated by using the computer program, GLOBAL79, developed by Crump and Watson (1979). At low doses, upper 95% confidence limits on the extra risk and lower 95% confidence limits on the dose producing a given risk are determined from a 95% upper confidence limit, q_1^*, on parameter q_1. Whenever $q_1 > 0$, at low doses the extra risk $P_t(d)$ has approximately the form $P_t(d) = q_1^* \times d$. Therefore, $q_1^* \times d$ is a 95% upper confidence limit on the extra risk and R/q_1^* is a 95% lower confidence limit on the dose, producing an extra risk of R. Let L_0 be the maximum value of the log-likelihood function. The upper-limit q_1^* is calculated by increasing q_1 to a value q_1^* such that when the log-likelihood is remaximized subject to this fixed value q_1^* for the linear coefficient, the resulting maximum value of the log-likelihood L_1 satisfies the equation

$$2\,(L_0 - L_1) = 2.70554$$

where 2.70554 is the cumulative 90% point of the chi-square distribution with one degree of freedom, which corresponds to a 95% upper-limit (one-sided). This approach of computing the upper confidence limit for the extra risk $P_t(d)$ is an improvement on the Crump et al. (1977) model. The upper confidence limit for the extra risk calculated at low doses is always linear. This is conceptually consistent with the linear non-threshold concept discussed earlier. The slope, q_1*, is taken as an upper-bound of the potency of the chemical in inducing cancer at low doses. [In the section calculating the risk estimates, $P_t(d)$ will be abbreviated as P.]

In fitting the dose-response model, the number of terms in the polynomial is chosen equal to (h-1), where h is the number of dose groups in the experiment, including the control group.

Whenever the multistage model does not fit the data sufficiently well, data at the highest dose is deleted and the model is refit to the rest of the data. This is continued until an acceptable fit to the data is obtained. To determine whether or not a fit is acceptable, the chi-square statistic

$$X^2 = \sum_{i=1}^{h} \frac{(X_i - N_i P_i)^2}{N_i P_i (1-P_i)}$$

is calculated where N_i is the number of animals in the i^{the} dose group, X_i is the number of animals in the i^{the} dose group with a tumor response, P_i is the probability of a response in the i^{the} dose group estimated by fitting the multistage model to the data, and h is the number of remaining groups. The fit is determined to be unacceptable whenever X^2 is larger than the cumulative 99% point of the chi-square distribution with f degrees of freedom, where f equals the number of dose groups minus the number of non-zero multistage coefficients.

12.3.5.6.1.2. Selection of Data. For some chemicals, several studies in different animal species, strains and sexes, each run at several doses and different routes of exposure, are available. A choice must be made as to which of the data sets from several studies to use in the model. It may also be appropriate to correct for metabolism differences between species and for absorption factors via different routes of administration. The procedures used in evaluating these data are consistent with the approach of making a maximum-likely risk estimate. They are as follows:

1. The tumor incidence data are separated according to organ sites or tumor types. The set of data (i.e., dose and tumor incidence) used in the model is the set where the incidence is statistically significantly higher than the control for at least one test dose level and/or where the tumor incidence rate shows a statistically significant trend with respect to dose level. The data set that gives the highest estimate of the lifetime carcinogenic risk, q_1^*, is selected in most cases. However, efforts are made to exclude data sets that produce spuriously high risk estimates because of a small number of animals. That is, if two sets of data show a similar dose-response relationship, and one has a very small sample size, the set of data having the larger sample size is selected for calculating the carcinogenic potency.
2. If there are two or more data sets of comparable size that are identical with respect to species, strain, sex and tumor sites, the geometric mean of q_1^*, estimated from each of these data sets, is used for risk assessment. The geometric mean of numbers A_1, A_2, ..., A_m is defined as

$$(A_1 \times A_2 \times \ldots \times A_m)^{1/m}.$$

3. If two or more significant tumor sites are observed in the same study, and if the data are available, the number of animals with at least one of the specific tumor sites under consideration is used as incidence data in the model.

12.3.5.6.1.3. **Calculation of Human Equivalent Dosages.** Following the suggestion of Mantel and Schneiderman (1975), it is assumed that mg/surface area/day is an equivalent dose between species. Since, to a close approximation, the surface area is proportional to the two-thirds power of the weight, as would be the case for a perfect sphere, the exposure in mg/day$^{2/3}$ of the weight is also considered to be equivalent exposure. In an animal experiment, this equivalent dose is computed in the following manner.

Let

L_e = duration of experiment

l_e = duration of exposure

m = average dose per day in mg during administration of the agent (i.e., during l_e), and

W = average weight of the experimental animal

Then, the lifetime exposure is

$$d = \frac{l_e \times m}{L_e \times W^{2/3}}$$

ORAL: Often exposures are not given in units of mg/day, and it becomes necessary to convert the given exposures into mg/day. Similarly, in drinking water studies, exposure is expressed as ppm in the water. For example, in most feeding studies exposure is given in terms of ppm in the diet. In these cases, the exposure in mg/day is

$$m = \text{ppm} \times F \times r$$

where ppm is parts per million of the carcinogenic agent in the diet or water, F is the weight of the food or water consumed per day in kg, and r is the absorption fraction. In the absence of any data to the contrary, r is assumed to be equal to one. For a uniform diet, the weight of the food consumed is proportional to the calories required, which in turn is proportional to the surface area, or two-thirds power of the weight. Water demands are also assumed to be proportional to the surface area, so that

$$m \propto ppm \times W^{2/3} \times r$$

or

$$\frac{m}{rW^{2/3}} \propto ppm.$$

As a result, ppm in the diet or water is often assumed to be an equivalent exposure between species. However, this is not justified for the present study, since the ratio of calories to food weight is very different in the diet of man as compared to laboratory animals, primarily due to differences in the moisture content of the foods eaten. For the same reason, the amount of drinking water required by each species also differs. It is therefore necessary to use an empirically-derived factor, $f = F/W$, which is the fraction of an organism's body weight that is consumed per day as food, expressed as follows:

		Fraction of Body Weight Consumed as	
Species	W	f_{food}	f_{water}
Man	70	0.028	0.029
Rats	0.35	0.05	0.078
Mice	0.03	0.13	0.17

Thus, when the exposure is given as a certain dietary or water concentration in ppm, the exposure in $mg/W^{2/3}$ is

$$\frac{m}{rW^{2/3}} = \frac{ppm \times F}{W^{2/3}} = \frac{ppm \times f \times W}{W^{2/3}} = ppm \times f \times W^{1/3}$$

When exposure is given in terms of mg/kg/day = m/Wr = s, the conversion is simply

$$\frac{m}{rW^{2/3}} = s \times W^{1/3}.$$

INHALATION: When exposure is via inhalation, the calculation of dose can be considered for two cases where 1) the carcinogenic agent is either a completely water-soluble gas or an aerosol and is absorbed proportionally to the amount of air breathed in, and 2) where the carcinogen is a poorly water-soluble gas which reaches an equilibrium between the air breathed and the body compartments. After equilibrium is reached, the rate of absorption of these agents is expected to be proportional to the metabolic rate, which in turn is proportional to the rate of oxygen consumption, which in turn is a function of surface area.

Case 1: Agents that are in the form of particulate matter or virtually completely absorbed gases, such as sulfur dioxide, can reasonably be expected to be absorbed proportionally to the breathing rate. In this case the exposure in mg/day may be expressed as

$$m = I \times v \times r$$

where I = inhalation rate per day in m³, v = mg/m³ of the agent in air, and r = the absorption fraction.

The inhalation rates, I, for various species can be calculated from the observations of the Federation of American Societies for Experimental Biology (FASEB, 1974) that 25 g mice breathe 34.5 ℓ/day and 113 g rats breathe 105 ℓ/day. For mice and rats of other weights, W (in kg), the surface area proportionality can be used to find breathing rates in m³/day as follows:

$$\text{For mice, } I = 0.0345\ (W/0.025)^{2/3}\ \text{m}^3/\text{day}$$

$$\text{For rats, } I = 0.105\ (W/0.113)^{2/3}\ \text{m}^3/\text{day}$$

For humans, the value of 20 m³/day* is adopted as a standard breathing rate (ICRP, 1977). The equivalent exposure in mg/$W^{2/3}$ for these agents can be derived from the air intake data in a way analogous to the food intake data. The empirical factors for the air intake/kg/day, $i = I/W$, based upon the previously stated relationships, are tabulated as follows:

Species	W	i = I/W
Man	70	0.29
Rats	0.35	0.64
Mice	0.03	1.3

Therefore, for particulates or completely absorbed gases, the equivalent exposure in mg/$W^{2/3}$ is

$$d = \frac{m}{W^{2/3}} = \frac{Ivr}{W^{2/3}} = \frac{iWvr}{W^{2/3}} = iW^{1/3}vr$$

In the absence of experimental information or a sound theoretical argument to the contrary, the fraction absorbed, r, is assumed to be the same for all species.

Case 2: The dose in mg/day of partially soluble vapors is proportional to the O_2 consumption, which in turn is proportional to $W^{2/3}$ and is also proportional to the solubility of the gas in body fluids, which can be expressed as an absorption coefficient, r, for the gas. Therefore, expressing the O_2 consumption as $O_2 = k\ W^{2/3}$, where k is a constant independent of species, it follows that

$$m = k\ W^{2/3} \times v \times r$$

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*From "Recommendation of the International Commission on Radiological Protection", page 9. The average breathing rate is 10^7 cm³ per 8-hour workday and 2×10^7 cm³ in 24 hours.

or

$$d = \frac{m}{W^{2/3}} = kvr$$

As with Case 1, in the absence of experimental information or a sound theoretical argument to the contrary, the absorption fraction, r, is assumed to be the same for all species. Therefore, for these substances a certain concentration in ppm or $\mu g/m^3$ in experimental animals is equivalent to the same concentration in humans. This is supported by the observation that the minimum alveolar concentration necessary to produce a given "stage" of anesthesia is similar in man and animals (Dripps et al., 1977). When the animals are exposed via the oral route and human exposure is via inhalation or vice versa, the assumption is made, unless there is pharmacokinetic evidence to the contrary, that absorption is equal by either exposure route.

12.3.5.6.1.4. Calculation of the United Risk from Animal Studies. The risk associated with d $mg/kg^{2/3}$/day is obtained from GLOBAL79 and, for most cases of interest to risk assessment, can be adequately approximated by $P(d) = 1 - \exp(-q_1{}^*d)$. A "unit risk" in units X is simply the risk corresponding to an exposure of X = 1. This value is estimated simply by finding the number of $mg/kg^{2/3}$/day that corresponds to one unit of X, and substituting this value into the above relationship. Thus, for example, if X is in units of $\mu g/m^3$ in the air, then for Case 1, $d = 0.29 \times 70^{1/3} \times 10^{-3}$ $mg/kg^{2/3}$/day, and for Case 2, d = 1, when $\mu g/m^3$ is the unit used to compute parameters in animal experiments.

If exposures are given in terms of ppm in air, the following calculation may be used:

$$1 \text{ ppm} = 1.2 \times \frac{\text{molecular weight (gas)}}{\text{molecular weight (air)}} \text{ mg/m}^3$$

Note that an equivalent method of calculating unit risk would be to use mg/kg for the animal exposures, and then to increase the j^{the} polynomial coefficient by an amount

$$(W_h/W_a)^{j/3} \quad j = 1, 2, \ldots, k,$$

and to use mg/kg equivalents for the unit risk values.

ADJUSTMENTS FOR LESS THAN LIFESPAN DURATION OF EXPERIMENT: If the duration of experiment L_e is less than the natural lifespan of the test animal L, the slope q_1^*, or more generally the exponent g(d), is increased by multiplying a factor $(L/L_e)^3$. We assume that if the average dose d is continued, the age-specific rate of cancer will continue to increase as a constant function of the background rate. The age-specific rates for humans increase at least by the third power of the age and often by a considerably higher power, as demonstrated by Doll (1971). Thus, it is expected that the cumulative tumor rate would increase by at least the third power of age. Using this fact, it is assumed that the slope q_1^*, or more generally the exponent g(d), would also increase by at least the third power of age. As a result, if the slope q_1^* [or g(d)] is calculated at age L_e, it is expected that if the experiment had been continued for the full lifespan L at the given average exposure, the slope q_1^* [or g(d)] would have been increased by at least $(L/L_e)^3$.

This adjustment is conceptually consistent with the proportional hazard model proposed by Cox (1972) and the time-to-tumor model considered by Daffer et al. (1980), where the probability of cancer by age t and at dose d is given by

$$P(d,t) = 1 - \exp\left[-f(t) \times g(d)\right].$$

12.3.5.6.2. Unit Risk Estimates --

12.3.5.6.2.1. Data Available for Potency Calculation. Hexachlorobenzene has been shown to induce tumors in hamsters, mice and rats. The primary target organ appears to be the liver in all three of these species. Liver haemangioendotheliomas in hamsters and hepatocellular carcinomas in rats were significantly increased in the hexachlorobenzene-treated animals. The potency estimate calculated on the basis of hepatocellular carcinomas in female rats is used to derive unit risk estimates for hexachlorobenzene in air and water. This particular tumor site is selected for calculating unit risks because it is a malignant tumor in the primary target organ and results in the highest potency estimate.

Increased incidences of thyroid, parathyroid, adrenal and kidney tumors were also observed among these species. Fourteen data sets showing significant tumor incidences have been used herein to calculate the carcinogenic potency of hexachlorobenzene. These calculations provide a range of estimates that, in part, reflect the uncertainties inherent in the risk assessment process. Tables 12-31 through 12-34 summarize the data used to calculate the potency of hexachlorobenzene. These data have been discussed and evaluated elsewhere in this chapter.

12.3.5.6.2.2. Choice of Low-Dose Extrapolation. In addition to the multistage model currently used by CAG for low-dose extrapolation, CAG also uses three other models, the probit, the Weibull and the one-hit models, to estimate the risks from exposure to hexachlorobenzene using the data for hepatocellular carcinoma in female rats. These models cover almost the entire spectrum of risk estimates that could be generated from the existing mathematical extrapolation models. These models are generally statistical in character, and are not derived from biological arguments, except for the

TABLE 12-31

Tumor Incidences in Male and Female Hamsters Given Hexachlorobenzene in Diet[a]

Dose[b] (mg/kg/day)	Thyroid	Hepatoma		Liver Hemangioendothelioma	
	Male	Male	Female	Male	Female
0	0/40	0/40	0/30	0/40	0/39
4	0/30	14/30	14/30	1/30	0/30
9	1/30	26/30	17/30	6/30	2/30
16	8/57	49/57	51/60	20/57	7/60

[a] Source: Cabral et al., 1977

[b] If mg/surface area/day is assumed to be equivalent between humans and animals, the dose in mg/kg/day is multiplied by a factor $(0.1/70)^{1/3}$, where 70 and 0.1 kg are, respectively, the average body weights of humans and hamsters.

TABLE 12-32

Incidence of Liver Cell Tumors in Male and Female Swiss Mice Given Hexachlorobenzene Diet[a]

Dose[b] (mg/kg/day)	Male[c]	Female[c]
0	0/47	0/49
6	0/30	0/30
12	3/12	3/12
24	7/29	14/26

[a] Source: Cabral et al., 1979

[b] If the equivalent dose between humans and mice is assumed to be on the basis of body surface area, the dose in mg/kg/day is multiplied by a factor $(0.035/70)^{1/3}$, where 0.035 kg and 70 kg are, respectively, the average body weights of mice and humans.

[c] The number of animals that survived at the first observed liver cell tumor is used as the denominator.

TABLE 12-33

Liver and Kidney Tumor Incidence Rates in Male and Female Sprague-Dawley Rats Given Hexachlorobenzene in Diet[a]

Sex	Dose[b] (mg/kg/day)	Hepatocellular Carcinoma	Hepatoma	Renal Cell Adenoma
Male	0	0/54	0/54	7/54
	4.24	3/52	10/52	41/52
	8.48	4/56	11/56	42/56
Female	0	0/52	0/52	1/52
	4.67	36/56	26/56	7/56
	9.34	48/55	35/55	15/54

[a] Source: Lambrecht, 1983a,b. Additional data from this study on adrenal pheochromocytoma has recently become available (Peters et al., 1983, summarized in Table 12-26) but was not available when quantitative estimates were made.

[b] The dosages are calculated by the investigator based on the average food consumption of 22.6 g/rat/day and an average body weight of 400 g for male rats. For female rats, the average food consumption is 16.5 g/rat/day and the average body weight is 265 g. If the equivalent dose between humans and mice is assumed to be on the basis of body surface area, the dose presented in the table is multiplied by a factor $(W_a/70)^{1/3}$, where W_a is the body weight of male or female rats, and 70 kg is the human body weight.

TABLE 12-34

Incidence Rate of Adrenal Pheochromocytoma in Female Sprague-Dawley Rats (F_1 generation) in a 2-Generation Feeding Study

Dose[a] (mg/kg/day)	Incidence Rate[b] (used in calculations)	Revised Incidence Rate[c]
0	2/48	2/49
0.02	4/50	4/49
0.08	4/50	
0.40	5/49	4/49
1.90	17/49	

[a] If the equivalent dose between humans and rats is assumed to be on the basis of body surface, the dose in this table is multiplied by a factor $(0.35/70)^{1/3}$, where 70 kg and 0.35 kg are, respectively, assumed to be the body weight of humans and rats.

[b] Source: Arnold et al., 1983

[c] Source: Arnold, 1984. The amended 1984 data are presented in Table 12-28, but were not available when quantitative estimates were made.

multistage model which has been used to support the somatic mutation hypothesis of carcinogenesis (Armitage and Doll, 1954; Whittemore, 1978; Whittemore and Keller, 1978). The main differences among these models is the rate at which the response function, P(d), approaches zero or P(0) as dose, d, decreases. For instance, the probit model would usually predict a smaller risk at low doses than the multistage model because of the difference of the decreasing rate in the low-dose region. However, it should be noted that one could always artificially give the multistage model the same (or even greater) rate of decrease as the probit model by making some dose transformation and/or by assuming that some of the parameters in the multistage model are zero. This, of course, is not reasonable without knowing, a priori, what the carcinogenic process for the agent is. Although the multistage model appears to be the most reasonable or at least the most general model to use, the point estimate generated from this model is of limited value because it does not help to determine the shape of the dose-response curve beyond experimental exposure levels. furthermore, point estimates at low doses extrapolated beyond experimental doses could be extremely unstable and could differ drastically, depending on the amount of the lowest experimental dose. Since upper-bound estimates from the multistage model at low doses are relatively more stable than point estimates, it is suggested that the upper-bound estimate for the risk (or the lower-bound estimate for the dose) be used in evaluating the carcinogenic potency of a suspect carcinogen. The upper-bound estimate can be taken as a plausible estimate if the true dose-response curve is actually linear at low doses. The upper-bound estimate means that the risks are not likely to be higher, but could be lower if the compound has a concave upward dose-response curve or a threshold at low doses. Another reason one can, at best, obtain an upper-bound estimate of the risk when animal data are used is that the estimated risk is a probability conditional to the assumption that an animal carcinogen is also a human carcinogen. Therefore, in reality, the actual risk could range from a value near zero to an upper-bound estimate.

12.3.5.6.2.3. Calculation of the Carcinogenic Potency of Hexachlorobenzene. Fourteen sets of tumor incidences which show significant increases (see Tables 12-31 through 12-34) are used herein to calculate the carcinogenic potency of hexachlorobenzene. Since preparing these calculations additional data from the Lambrecht et al. (1983a,b) study (adrenal pheochromocytoma) and from the Arnold et al. (1983) study (neoplastic liver nodules) have become available. Quantitative estimates have not been made using this data. Using the multistage model for low-dose extrapolation, as shown in Table 12-35, the potency estimates calculated on the basis of these data sets are approximately within an order of magnitude from each other, with the exception of the thyroid tumor. These potencies provide a range of estimates that reflects the uncertainties stemming from the differences in species, tumor sites, solvent vehicles and composition of diet. The range does not reflect uncertainty resulting from the use of different extrapolation models.

To calculate the unit risks of hexachlorobenzene in air and water, CAG used an estimate of carcinogenic potency based upon the data for hepatocellular carcinoma in female rats. For comparison, three additional low-dose extrapolation models, the probit, the Weibull and the one-hit models, are also used to provide risk estimates at dose levels 0.01, 0.1 and 1 mg/kg/day. These results are presented in Table 12-36. The maximum likelihood estimate of the parameters for all four models are presented in Table A-1 in the Appendix. At 1 mg/kg/day, all four models predict comparable risks.

TABLE 12-35

The Carcinogenic Potency[a] of Hexachlorobenzene, Calculated on the Basis of 14 Data Sets,[b] Using the Linearized Multistage Model

Study	Date Base	Dose is Assumed to be Equivalent on the Basis of		Reference
		Body Weight	Surface Area	
Hamster	Thyroid (male)	9.3×10^{-3}	8.3×10^{-2}	Cabral
	Hepatoma:			et al., 1977
	Male	1.9×10^{-1}	1.7	
	Female	1.5×10^{-1}	1.3	
	Hemangioendothelioma:			
	Male	3.2×10^{-2}	2.8×10^{-1}	
	Female	1.1×10^{-2}	1.0×10^{-1}	
Mice	Liver cell:			Cabral
	Male	1.7×10^{-2}	2.1×10^{-1}	et al., 1979
	Female	1.4×10^{-2}	1.8×10^{-1}	

TABLE 12-35 (cont.)

Study	Date Base	Dose is Assumed to be Equivalent on the Basis of		Reference
		Body Weight	Surface Area	
Rats	Renal cell:			Lambrecht, 1983
	Male	2.5×10^{-1}	1.4	
	Female	4.2×10^{-2}	2.6×10^{-1}	
	Hepatocellular carcinoma:			
	Male	1.8×10^{-2}	1.0×10^{-1}	
	Female	2.7×10^{-1}	1.7	
	Hepatoma:			
	Male	4.7×10^{-2}	2.6×10^{-1}	
	Female	1.5×10^{-1}	9.0×10^{-1}	
Rats 2-generation study	Adrenal Pheochromocytoma (female)	2.8×10^{-1}	1.6	Arnold et al., 1983

[a] q_1^* $(mg/kg/day)^{-1}$ is the 95% upper confidence limit of the linear component in the multistage model.

[b] Since preparing these calculations, additional data from Lambrecht et al. (1983a,b) study (adrenal pheochromocytoma) and from Arnold et al. (1983) study (neoplastic liver nodules) has become available. These data have not been evaluated.

TABLE 12-36

Upper-Bound[a] (Point) Estimation of Risk, Based on Hepatocellular Carcinoma in Female Rats[b]

Assumption of Human Equivalent Dose	Models	Risk at Dose Level (mg/kg/day)		
		0.01	0.1	1.00
On the basis of body weight	multistage	2.7×10^{-3} (2.2×10^{-3})	2.7×10^{-2} (2.2×10^{-2})	2.4×10^{-1} (2.0×10^{-1})
	probit	3.6×10^{-9} (1.3×10^{-10})	1.0×10^{-3} (8.9×10^{-5})	3.4×10^{-1} (1.2×10^{-1})
	Weibull	1.2×10^{-2} (2.5×10^{-3})	8.4×10^{-2} (2.5×10^{-2})	4.3×10^{-1} (2.2×10^{-1})
	one-hit	2.7×10^{-3} (2.2×10^{-3})	2.7×10^{-2} (2.2×10^{-2})	2.4×10^{-1} (2.0×10^{-1})
On the basis of surface area	multistage	1.7×10^{-2} (1.4×10^{-2})	1.7×10^{-1} (1.3×10^{-1})	8.0×10^{-1} (7.4×10^{-1})
	probit	6.2×10^{-5} (4.1×10^{-6})	1.3×10^{-1} (2.9×10^{-2})	8.2×10^{-1} (7.5×10^{-1})
	Weibull	5.0×10^{-2} (1.3×10^{-2})	2.9×10^{-1} (1.3×10^{-1})	8.1×10^{-1} (7.4×10^{-1})
	one-hit	1.7×10^{-2} (1.4×10^{-2})	1.7×10^{-1} (1.3×10^{-1})	8.0×10^{-1} (7.4×10^{-1})

[a] 95% upper confidence limit

[b] Source: Lambrecht, 1983

At lower doses, the multistage model predicts a higher risk than the probit model, but a lower risk than the Weibull model.

12.3.5.6.2.4. **Risk Associated with 1 μg/ℓ of Hexachlorobenzene in Drinking Water.** Under the assumption that daily water consumption for a 70 kg person is 2 ℓ, the hexachlorobenzene intake in terms of mg/kg/day is

$$d = 2\ \ell \times 1\ \mu g/\ell \times 10^{-3}\ mg/\ell/70\ kg = 2.86 \times 10^{-5}\ mg/kg/day.$$

Therefore, the risk from drinking water containing 1 μg/ℓ of hexachlorobenzene is estimated to be

$$P = 1.7 \times 2.86 \times 10^{-5} = 4.9 \times 10^{-5}.$$

This calculation uses the carcinogenic potency $q_1^* = 1.7/(mg/kg/day)$, based on the data on hepatocellular carcinomas in female rats, assuming that dose per surface area is equivalent between rats and humans. If the equivalent dose is assumed to be on the basis of body weight, the unit risk, P, would be reduced to 7.6×10^{-6}.

12.3.5.6.2.5. **Risk Associated with 1 μg/m³ of Hexachlorobenzene in Air.** Since no inhalation study has been performed on hexachlorobenzene, the risk from inhalation exposure can only be estimated by using the carcinogenic potency, $q_1^* = 1.7/(mg/kg/day)$, as calculated from the dietary study referred to elsewhere in this chapter. The assumption is made that the hexachlorobenzene absorption rate is the same whether exposure is via the oral or the inhalation route.

Assuming the volumetric breathing rate of 20 m³/day for a 70 kg person, the rate in mg/kg/day corresponding to 1 μg/m³ hexachlorobenzene in air is

$$d = (20\ m^3/day) \times (10^{-3}\ mg/\mu g) \times (1/70\ kg) = 2.86 \times 10^{-4}\ mg/kg/day.$$

Therefore, the risk due to inhaling air contaminated with 1 μg/m³ hexachlorobenzene is

$$P = 1.7 \times 2.86 \times 10^{-4} = 4.9 \times 10^{-4}.$$

This estimation is based on the assumption that dose per surface area is equivalent between humans and rats. If dose per body weight is assumed to be equivalent, the unit risk would be reduced to 7.6×10^{-5}.

12.3.5.6.3. Comparison of Potency with Other Compounds -- One of the uses of quantitative potency estimates is to compare the relative potency of carcinogens. Figure 12-1 is a histogram representing the frequency distribution of potency indices for 54 suspect carcinogens evaluated by CAG. The actual data summarized by the histogram are presented in Table 12-37. The potency index is derived from q_1^*, the 95% upper bound of the linear component in the multistage model, and is expressed in terms of $(\text{mMol/kg/day})^{-1}$. Where no human data were available, animal oral studies were used in preference to animal inhalation studies, since oral studies have constituted the majority of animal studies.

Based on data concerning hepatocellular carcinomas in female rats, the potency index for hexachlorobenzene has been calculated as 5×10^{2}. This figure is derived by multiplying the slope $q_1^* = 1.7/(\text{mg/kg/day})$ and the molecular weight of hexachlorobenzene, 284.4. This places the potency index for hexachlorobenzene in the second quartile of the 54 suspect carcinogens evaluated by CAG.

The ranking of relative potency indices is subject to the uncertainties involved in comparing a number of potency estimates for different chemicals based on varying routes of exposure in different species by means of data from studies whose quality varies widely. All of the indices presented are based on estimates of low-dose risk, using linear extrapolation from the observational range. These indices may not be appropriate for the comparison of potencies if linearity does not exist at the low-dose range, or if comparison is to be made at the high-dose range. If the latter is the case, then an index other than the one calculated above may be more appropriate.

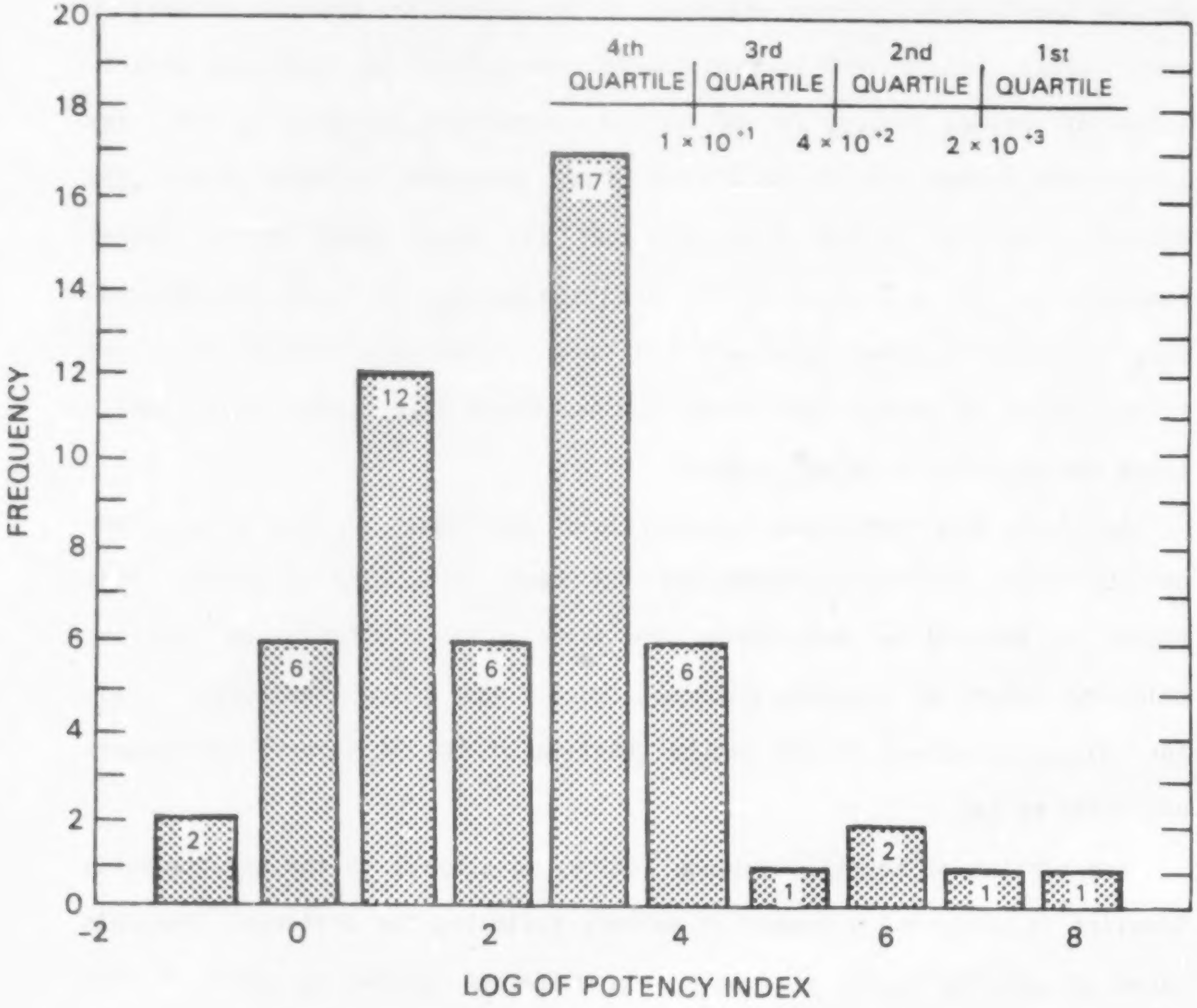

FIGURE 12-1

Histogram Representing the Frequency Distribution of the Potency Indices of 54 Suspect Carcinogens Evaluated by the Carcinogen Assessment Group

TABLE 12-37

Relative Carcinogenic Potencies Among 54 Chemicals Evaluated by the Carcinogen Assessment Group as Suspect Human Carcinogens[a,b,c]

Compounds	Slope $(mg/kg/day)^{-1}$	Molecular Weight	Potency Index	Order of Magnitude ($\log_{10}$ index)
Acrylonitrile	0.24 (W)	53.1	$1 \times 10^{+1}$	+1
Aflatoxin B_1	2924	312.3	$9 \times 10^{+5}$	+6
Aldrin	11.4	369.4	$4 \times 10^{+3}$	+4
Allyl chloride	1.19×10^{-2}	76.5	9×10^{-1}	0
Arsenic	15 (H)	149.8	$2 \times 10^{+3}$	+3
B[a]P	11.5	252.3	$3 \times 10^{+3}$	+3
Benzene	5.2×10^{-2} (W)	78	4×10^{0}	+1
Benzidene	234 (W)	184.2	$4 \times 10^{+4}$	+5
Beryllium	1.40	9	$1 \times 10^{+1}$	+1
Cadmium	6.65 (W)	112.4	$7 \times 10^{+2}$	+3
Carbon tetrachloride	1.30×10^{-1}	153.8	$2 \times 10^{+1}$	+1
Chlordane	1.61	409.8	$7 \times 10^{+2}$	+3
Chlorinated ethanes				
1,2-Dichloroethane	6.9×10^{-2}	98.9	7×10^{0}	+1
Hexachloroethane	1.42×10^{-2}	236.7	3×10^{0}	0
1,1,2,2-Tetrachloroethane	0.20	167.9	$3 \times 10^{+1}$	+1
1,1,1-Trichloroethane	1.6×10^{-3}	133.4	2×10^{-1}	-1
1,1,2-Trichloroethane	5.73×10^{-2}	133.4	8×10^{0}	+1
Chloroform	7×10^{-2}	119.4	8×10^{0}	+1
Chromium	41 (W)	100	$4 \times 10^{+3}$	+4

TABLE 12-37 (cont.)

Compounds	Slope $(mg/kg/day)^{-1}$	Molecular Weight	Potency Index	Order of Magnitude (log_{10} index)
DDT	8.42	354.5	$3x10^{+3}$	+3
Dichlorobenzidine	1.69	253.1	$4x10^{+2}$	+3
1,1-Dichloroethylene	1.47×10^{-1} (I)	97	$1x10^{+1}$	+1
Dieldrin	30.4	380.9	$1x10^{+4}$	+4
Dinitrotoluene	0.31	182	$6x10^{+1}$	+2
Diphenylhydrazine	0.77	180	$1x10^{+2}$	+2
Epichlorohydrin	9.9×10^{-3}	92.5	$9x10^{-1}$	0
Bis(2-chloroethyl)ether	1.14	143	$2x10^{+2}$	+2
Bis(chloromethyl)ether	9300 (I)	115	$1x10^{+6}$	+6
Ethylene dibromide (ECB)	8.51	187.9	$2x10^{+3}$	+3
Ethylene oxide	1.26 (I)	44.1	$6x10^{+1}$	+2
Heptachlor	3.37	373.3	$1x10^{+3}$	+3
Hexachlorobenzene	1.67	284.4	$5x10^{+2}$	+3
Hexachlorobutadiene	7.75×10^{-2}	261	$2x10^{+1}$	+1
Hexachlorocyclohexane				
Technical grade	4.75	290.9	$1x10^{+3}$	+3
Alpha isomer	11.12	290.9	$3x10^{+3}$	+3
Beta isomer	1.84	290.9	$5x10^{+2}$	+3
Gamma isomer	1.33	290.9	$4x10^{+2}$	+3
Hexachlorodibenzodioxin	$1.1 \times 10^{+4}$	391	$4x10^{+6}$	+7
Methylene chloride	6.3×10^{-4}	84.9	$5x10^{-2}$	-1
Nickel	1.15 (W)	58.7	$7x10^{+1}$	+2

TABLE 12-37 (cont.)

Compounds	Slope $(mg/kg/day)^{-1}$	Molecular Weight	Potency Index	Order of Magnitude ($\log_{10}$ index)
Nitrosamines				
Dimethylnitrosamine	25.9 (not by q_1^*)	74.1	$2 \times 10^{+3}$	+3
Diethylnitrosamine	43.5 (not by q_1^*)	102.1	$4 \times 10^{+3}$	+4
Dibutylnitrosamine	5.43	158.2	$9 \times 10^{+2}$	+3
N-nitrosopyrrolidine	2.13	100.2	$2 \times 10^{+2}$	+2
N-nitroso-N-ethylurea	32.9	117.1	$4 \times 10^{+3}$	+4
N-nitroso-N-methylurea	302.6	103.1	$3 \times 10^{+4}$	+4
N-nitroso-diphenylamine	4.92×10^{-3}	198	1×10^{0}	0
PCBs	4.34	324	$1 \times 10^{+3}$	+3
Phenols				
2,4,6-Trichlorophenol	1.99×10^{-2}	197.4	4×10^{0}	+1
Tetrachlorodibenzo-p-dioxin	1.56×10^{5}	322	$5 \times 10^{+7}$	+8
Tetrachloroethylene	3.5×10^{-2}	165.8	6×10^{0}	+1
Toxaphene	1.13	414	$5 \times 10^{+2}$	+3
Trichloroethylene	1.9×10^{-2}	131.4	2.5×10^{0}	0
Vinyl chloride	1.75×10^{-2} (I)	62.5	1×10^{0}	0

[a] Animal slopes are 95% upper-limit slopes based on the linearized multistage model. They are calculated based on animal oral studies, except for those indicated by I (animal inhalation), W (human occupational exposure, and H (human drinking water exposure). Human slopes are point estimates based on the linear non-threshold model.

[b] The potency index is a rounded-off slope in $(mMol/kg/day)^{-1}$ and is calculated by multiplying the slopes in $(mg/kg/day)^{-1}$ by the molecular weight of the compound.

[c] Not all of the carcinogenic potencies presented in this table represent the same degree of certainty. All are subject to change as new evidence becomes available.

12.3.5.6.4. **Summary of Quantitative Estimation** -- Data on hepatocellular carcinomas in female rats have been used to estimate the carcinogenic potency of hexachlorobenzene and the risks associated with one unit of the compound in drinking water and air. The upper bound cancer risks associated with 1 $\mu g/\ell$ of hexachlorobenzene in drinking water and 1 $\mu g/m^3$ of hexachlorobenzene in air are estimated to be 5×10^{-5} and 5×10^{-4}, respectively. These estimates are calculated on the basis of the assumption that dose per surface area is equivalent among species. If the dose is assumed to be equivalent on the basis of body weight, the corresponding risk would be reduced approximately by a factor of 6. The carcinogenic potencies of hexachlorobenzene are also estimated on the basis of 13 other data sets, encompassing different tumor sites and animal species. Except for the case of thyroid tumors, these potency estimates differ from each other within a single order of magnitude. The range of the estimates reflects the uncertainties due to differences in species, tumor sites, solvent vehicles, composition of diet, etc.

12.3.5.7. **CARCINOGENICITY SUMMARY** -- In a lifetime study of hexachlorobenzene dietary administration to hamsters, hepatoma was induced in both males and females. The response at a dose of 4-5 mg/kg/day was 47% for both sexes and controls had no hepatomas. In addition to hepatomas, hamsters responded to hexachlorobenzene treatment with malignant liver haemangioendothelioma and thyroid adenoma. The incidence of haemangioendothelioma was 20% in males (versus 0 in controls) at 8 mg/kg/day and 12% in females (versus 0 in controls) at 16 mg/kg/day. The thyroid adenoma occurred at 14% incidence in males treated with 16 mg/kg hexachlorobenzene (versus 0 in controls).

Liver cell tumors, described as hepatomas, were also produced in both sexes in Swiss mice. At 24 mg/kg/day the incidence was 34% for females and 16% for males and the response showed a dose-dependency not only in the number of tumor-bearing animals but also in the latent period, multiplicity and size of tumors. In ICR mice, hexachlorobenzene administered concurrently with polychlorinated terphenyl induced hepatocellular carcinomas.

In rats target organs for hexachlorobenzene-induced tumors included liver, kidney, adrenal gland and parathyroid gland in various studies. Liver tumors were found in three studies which included three different strains of rat: Agus (a liver tumor sensitive strain), Wistar and Sprague-Dawley rats. These tumors were induced with doses between 1.5 and 8 mg/kg/day. The incidence was as high as 100% in Agus rats but lower for the other strains. Renal cell tumors were found in one study on Sprague-Dawley rats. In two studies on Sprague-Dawley rats, significant increases in adrenal pheochromocytoma in females were found. In one of these studies the incidence of parathyroid tumors in males was significantly increased as well.

Table 12-38 summarizes the tumor data for hamsters, mice and rats for hexachlorobenzene experiments.

The data on hexachlorobenzene provide sufficient evidence of the carcinogenicity and tumorigenicity of hexachlorobenzene since there were increased incidences of malignant tumors of the liver in two species (haemangioendothelioma in hamsters and hepatocellular carcinoma in rats) as well as reports of hepatoma in mice, rats and hamsters.

The appearance of thyroid tumors in hamsters and adrenal pheochromocytomas and parathyroid tumors in rats as a result of hexachlorobenzene exposure is particularly interesting because of the clinical association of adrenal pheochromocytomas with parathyroid and thyroid tumors in humans (Fraumeni,

TABLE 12-38

Significantly Increased Incidence of Tumors in Animals Given Hexachlorobenzene in Diet

Animal (strain)	Organ	Tumor	% Treated/% Control		Lowest Dose to Produce Tumor (mg/kg bw/day)	Reference
			Males	Females		
Hamsters	liver	hepatoma	47/0	47/0	4	Cabral, 1977
Hamsters	liver	haemangioendothelioma	20/0	12/0	8 in males 16 in females	Cabral, 1977
Mice	liver	hepatoma	16/0	34/0	24	Cabral, 1979
Rats (S.D.)	liver	neoplastic nodules	NS	20/0	1.5	Arnold, 1983
Rats (S.D.)	liver	hepatoma	19/0	46/0	4-5	Lambrecht et al., 1983a
Rats (S.D.)	liver	hepatocellular carcinoma	NS	64/0	4-5	Lambrecht et al., 1983a
Rats (Wistar)	liver	hepatoma		67/0	6-8	Smith and Cabral, 1980
Rats (Agus)	liver	hepatoma		100/0	6-8	Smith and Cabral, 1980
Rats (S.D.)	adrenal	pheochromocytoma	NS	35/4	1.5	Arnold, 1983, 1984
Rats (S.D.)	adrenal	pheochromocytoma	NS	91/14	4-5	Peters et al., 1983
Rats (S.D.)	kidney	renal cell adenoma	79/13	13/2	4-5	Lambrecht et al., 1983b
Rats (S.D.)	parathyroid	adenoma	25/4	NS	1.5	Arnold, 1983
Hamsters	thyroid	adenoma	14/0		16	Cabral, 1977

NS = Not stated

1974; Hill, 1974), and because follow-up of individuals in Turkey, who were accidentally exposed to hexachlorobenzene over 25 years ago, shows a marked elevation in thyroid tumors. Only a few of these subjects have had their thyroid tumors examined histologically and the pathology reports are not yet available.

If the IARC criteria for the classification of carcinogens were used, this animal evidence would be considered "sufficient." In the absence of human evidence of carcinogenicity, hexachlorobenzene would be classed in IARC category 2B, meaning that it is probably carcinogenic in humans.

A quantitative estimate of the carcinogenic potency of hexachlorobenzene and an upper-bound estimate of the risks from continuous human exposures to 1 $\mu g/m^3$ in air and 1 $\mu g/\ell$ in drinking water were made from data on the hepatocellular carcinoma response in female rats. The upper-bound slope of the dose-response curve, q_1^*, is 1.7/(mg/kg/day), giving a potency index which is in the second quartile of 54 suspect carcinogens evaluated by the Carcinogen Assessment Group. The unit risks for air and water exposures are 4.9×10^{-4} for 1 $\mu g/m^3$ in ambient air and 4.9×10^{-5} for 1 $\mu g/\ell$ in drinking water. Corresponding estimates from 13 other data sets, encompassing different tumor sites and animal species, fall within a factor of 10 of these estimates except for thyroid tumors in hamsters, which give estimates of about 1/20 of the potency based on the rat hepatocellular carcinoma response.

12.3.6. **Reproductive and Teratogenic Effects.** Hexachlorobenzene has been shown to be transported via the placenta into fetal tissues and to be present in the milk of nursing dams (see Section 12.1.2.). The NOEL in a 4-generation reproduction study with rats was reported to be 20 ppm of hexachlorobenzene in the diet. Pups from treated dams (receiving diets containing 80 ppm hexachlorobenzene) recovered from elevated liver weights when nursed by foster dams. Hepatomegaly and reduced survival was reported in kittens from cats receiving 263 ppm of hexachlorobenzene in their diets. Infant rhesus monkeys developed clinical signs of toxicity, but histologic examination showed only mild effects. Fetal mice from dams treated with 100 mg/kg/day during days 7-16 of gestation exhibited teratogenic abnormalities.

Results from a 4-generation reproduction study with Sprague-Dawley rats was reported by Grant et al. (1977). Weanling rats, in groups of 20 females and 10 males, were fed diets containing 0, 10, 20, 40, 80, 160, 320 or 640 µg hexachlorobenzene/g and at 100 days of age the F_0 generation was mated to produce the F_{1a} generation. The F_{1a} pups were weaned at 21 days, and the F_0 rats were rested for 14 days and again mated to produce the second litter, F_{1b} animals. The F_{1b} animals were then used to produce the next generation, and this sequence was followed to the F_{4b} generation. The two highest doses (320 and 640 µg/g) were toxic to the mothers and resulted in 20 and 50% mortality, respectively, before the first whelping and 25% in each high dose group before the second whelping. In addition, the fertility index in these rats was greatly reduced in these two dose groups and the average litter size was decreased in the F_{1b}, F_{2a} and F_{2b} generations. The pups exhibited no gross abnormalities, but there was an increased number of stillbirths and all pups born alive died within 5 days in the 320 and 640 µg/g diet groups.

At the 160 µg/g level, 55% of the pups survived to day 5 but survival to day 21 was greatly reduced. The number of live births and survival was normal for the first two generations at the 80 µg/g dietary level, but by the third generation there were stillbirths and a low degree of postnatal viability. In addition, birth and weanling body weights were consistently less than those of the control group. At 40 µg/g diet only the liver weights of the 21-day-old pups were significantly increased, while the kidney, heart and brain weights were not affected. Tissue concentrations of hexachlorobenzene were dose-related, with body fat having the highest concentration. The NOEL was reported to be 20 ppm in the diet.

The effect of hexachlorobenzene on rat reproduction was also reported by Kitchin et al. (1982). Female Sprague-Dawley rats (10 animals/treatment group) were fed diets containing 0, 60, 80, 100, 120 and 140 µg hexachlorobenzene/g of diet. The females were mated with untreated males after 96 days and then bred a second time 12 days after weaning of the F_{1a} litter. Fertility and fecundity of treated females were not affected by treatment; however, a dose-related 21-day increase in mortality was observed in both litters and the LD_{50} values were determined to be 100 and 140 µg/g (maternal dietary concentration) for the F_{1a} and F_{1b} generation, respectively.

Mendoza et al. (1978) studied the effects of hexachlorobenzene on preweanling Wistar rats after a reciprocal transfer between 5 treated and 5 control dams. A significant increase in the liver weight over that of the control was observed in pups nursed by dams fed diets containing 80 µg hexachlorobenzene/g for 2 weeks before mating until birth, but this effect did not persist after the treated pups were transferred to a control foster dam. Similarly, the pups nursed by treated dams had smaller brains, hearts, kidneys and spleens than the controls, and these organs were larger in treated pups nursed by control dams. The authors concluded that hexachlorobenzene transmission via the milk had greater effects on the pups than transmission via the placenta.

Mendoza et al. (1979) placed female Wistar rats on diets containing 80 µg hexachlorobenzene/g beginning 2 weeks before mating until 35-36 days after weaning. Results indicated that there were no marked differences in the external appearance, body weight, liver weight, gestation, or neonatal survival between the hexachlorobenzene treated and control females. In addition, there were no differences in the number of litters, average number of pups/litter, average number of pups at birth and gestation index.

Hansen et al. (1979) studied the effects of hexachlorobenzene on reproduction in cats fed contaminated pork cakes for 142 days. These cakes contained 90±51 μg hexachlorobenzene/g, equivalent to an intake of 3 mg/day/cat, and were obtained from gilts fed diets containing 100 μg hexachlorobenzene/g for 6-8 weeks before slaughter. The positive and untreated control groups received pork cakes from gilts fed diets that did not contain hexachlorobenzene, with the positive control group receiving hexachlorobenzene-spiked cakes (263±120 μg/g equivalent to 8.7 mg/day/cat). These females were mated with untreated males and the resulting kittens did not receive hexachlorobenzene-containing cakes. Effects on survival were noted in kittens born to only those cats receiving hexachlorobenzene-spiked cakes and was apparently due to the kittens being too weak to survive the stress of weaning. There was a tendency for reduced average litter sizes and increased mortality of nursing kittens, and statistically significant hepatomegaly and reduction in kitten survival at weaning. Treated females exhibited a net weight loss and increased susceptibility to disease but no changes in relative organ weights, hematologic parameters, or fecal coproporphyrin excretion.

Rush et al. (1983) fed adult male and female standard dark minks (Mustela vision) diets containing 0, 1 or 5 ppm hexachlorobenzene and then mated the males to the females in each of the respective study groups. The resulting mink kits were fed their parents respective diets after weaning from their mothers. The effects of exposures to hexachlorobenzene _in utero_ and from nursing milk resulted in increased mortality in the hexachlorobenzene-treated weanlings with mortality in the 0, 1 and 5 ppm groups being 8.2, 44.1 and 77.4%, respectively. The surviving kits from all three groups had no observed alterations in whole body, kidney or liver weights and no observed damage to the kidneys or livers at 17 weeks of age. Induction of hepatic mixed-function oxidases was observed in the surviving hexachlorobenzene-exposed kits without any observable frank hepatotoxicity.

Bailey et al. (1980) studied the transfer of hexachlorobenzene to three nursing infant rhesus monkeys from three lactating mothers receiving by gavage 64 mg/kg/day of hexachlorobenzene suspended in methyl cellulose for 60 days. The hexachlorobenzene concentrated in the mothers' milk ranged from 7.51-186 ppm during the dosing schedule. One infant, by day 22, had developed symptoms of hypoactivity and lethargy which progressed to ataxia and death 1 week later. Autopsy revealed severely congested lungs. A second infant died on day 38 and autopsy revealed a subdural hematoma and bilateral hemorrhagic pneumonia. This indicated that the risk of exposure to nursing infants was greater than the risk to their mothers. Blood (0.42-49.44 ppm) and tissue levels in the infants were higher than in their mothers (0.41-16.16 ppm blood), and the infants developed clinical symptoms of toxicity while the mothers were asymptomatic.

Studies on the placental transfer of hexachlorobenzene in Wistar rats and New Zealand rabbits did not reveal any apparent adverse effects on fetal development. The female rats were dosed daily with 5, 10, 20, 40 or 80 mg/kg from day 6-16 of gestation, whereas the rabbits were treated with 0, 0.1, 1.0 or 10 mg/kg from day 1-27 of gestation. The compound was dissolved in corn oil and administered by means of a stomach tube (Villeneuve et al., 1974; Villeneuve and Hierlihy, 1975).

Khera (1974) conducted a teratogenicity study with groups of 7-16 female Wistar rats given single oral doses of 0, 10, 20, 40, 60, 80 or 120 mg hexachlorobenzene/kg suspended in corn oil or 0.25% aqueous gum tragacanth during gestation days 6-21. Maternal toxicity and reduction in fetal weights resulted from the two higher doses. Maternal toxicity was characterized by loss in body weight, hyperesthesia, tremors and convulsions. A significant increase in the incidence of unilateral and bilateral 14th rib was observed and was related to the duration of treatment (days 10-13, 6-16 or 6-21 of gestation) and the dose. Sternal defects were observed in only 1 of 4 experiments, which lead the authors to conclude that it is doubtful that hexachlorobenzene was the cause of the observed sternal defects. There were no hexachlorobenzene-related effects on external morphology. Visceral abnormalities were not observed, and microscopic examinations did not reveal any treatment-related change in the histology of the fetuses. Values for live and dead fetuses, resorption sites, and fetal weight were within the control limits.

Courtney et al. (1976) studied the effects of ingestion of 100 mg/kg/day hexachlorobenzene on days 7-16 of gestation in 10 pregnant CD-1 mice. This study was undertaken to evaluate the possibility that hexachlorobenzene could be responsible for fetal malformations seen in pregnant animals exposed to hexachlorobenzene-contaminated pentachloronitrobenzene. The results showed that the hexachlorobenzene-treated mice had significantly increased maternal liver-to-body weight ratios and decreased fetal body weights. Also, a significant increase in the incidence of abnormal fetuses per litter were observed as compared to control mice. The abnormalities that were observed in these affected fetuses were cleft palates, one straight leg, small kidneys, one renal agenesis, and enlarged renal pelvis.

They concluded from this study that the teratogenic activity of contaminated pentachloronitrobenzene was probably due to hexachlorobenzene.

12.4. INTERACTIONS

Certain chemicals have been shown to alter the toxicity and pharmacokinetics of hexachlorobenzene in mammals. Pentachlorophenol and iron increased the porphyrinogenic effect of hexachlorobenzene, whereas decachlorobiphenyl had no effect. Hexachlorobenzene pretreatment resulted in increased CCl_4 toxicity and altered immune responses in hexachlorobenzene-treated animals. In addition, hexachlorobenzene has been shown to induce hepatic xenobiotic metabolism and thus has the potential to alter the rate of metabolism of other chemicals (see Section 12.3.1.).

Debets et al. (1980b) studied the effect of pentachlorophenol (PCP) on hexachlorobenzene toxicity. Groups of female rats were fed diets containing 1000 μg hexachlorobenzene/g, 500 μg pentachlorophenol/g, or both chemicals in the same amounts, and a fourth group served as the control. Pentachlorophenol accelerated the onset of hexachlorobenzene-induced porphyria, as indicated by an increase in urinary excretion of uroporphyrin and a decrease of porphyrins with two and three carboxylic groups. This increase occurred ~3 weeks earlier in the hexachlorobenzene plus pentachlorophenol-treated animals than in hexachlorobenzene-treated animals.

Razzardini and Smith (1982) investigated diethylstilboestrol (DES) pretreatment on hexachlorobenzene metabolite excretion in young male and female F344/N rats. The rats were injected i.p. with four doses of DES dipropionate 20 μmoles/kg dissolved in arachis oil over a 24-day period and then given 14 mg/kg hexachlorobenzene by oral intubation for 7 days. The results indicated that the DES pretreatment stimulated the excretion, via urine and feces, in both males and females (Table 12-39).

TABLE 12-39

Analysis of the Excreta from Rats Administered Hexachlorobenzene After an Initial Treatment with Diethylstilboestrol[a,b]

Sex and Treatment	Pentachlorophenol	Tetrachlorobenzene-1,4-diol (nmole/24 hours/kg bw)	Pentachlorothiophenol
Urine			
Male + oil	151 ± 19	3 ± 1	23 ± 3
Male + DES	190 ± 22	17 ± 2[c]	158 ± 9[c]
Female + oil	174 ± 17	16 ± 2[d]	142 ± 12[e]
Female + DES	453 ± 105[f]	35 ± 9	176 ± 7[f]
Feces			
Male + oil	85 ± 15	Trace	74 ± 23
Male + DES	160 ± 23[f]	Trace	166 ± 33
Female + oil	116 ± 35	Trace	65 ± 4
Female + DES	279 ± 80	Trace	149 ± 13[c]

[a] Source: Razzardinia and Smith, 1982

[b] Male and female rats (52-54 and 71-73 days old, respectively) were given 20 μmole of DES dipropionate/kg dissolved in arachis oil (10 mg/mℓ) or oil alone by i.p. injection on days 1, 4, 14 and 24. From day 25 all rats were given 14 mg of hexachlorobenzene/kg by oral intubation daily for 7 days. After the last dose 24-hour samples of urine and feces were collected, hydrolyzed and analyzed. Results are means ± S.E.M. (n=4/group).

[c] Significance of differences from rats not given DES, $p<0.001$

[d] Significance of differences from males, $p<0.005$

[e] Significance of differences from males, $p<0.001$

[f] Significance of differences from rats not given DES, $p<0.05$

Total excretions of these metabolites were: male, 336±57; male + DES, 691±70 ($p<0.01$); female, 513±62; female + DES, 1092±175 ($p<0.025$) nmole/24 hours/kg

Blekkenhorst et al. (1980) reported that the simultaneous i.m. administration of iron and hexachlorobenzene caused a marked potentiation of hexachlorobenzene porphyrinogenic effect in rats. This was shown by a decrease in hepatic uroporphyrinogen decarboxylase activity and increased urinary and fecal porphyrin excretion. Conversely, simultaneous bleeding of hexachlorobenzene-treated rats diminished the porphyrinogenic effect of hexachlorobenzene.

Goldstein et al. (1978) studied the comparative toxicity of pure hexachlorobenzene (purity >99%) and technical hexachlorobenzene (purity 92%) which contained 200 ppm of decachlorobiphenyl and 4 ppm of octachlorodibenzofuran, in female CD rats fed diets containing 0, 30, 100, 300 or 1000 µg hexachlorobenzene/g for up to 15 weeks. Neither grade contained other chlorinated dibenzofurans or dibenzo-p-dioxins. Both grades resulted in comparable effects (porphyria, cutaneous lesions, hyperexcitability, changes in liver enzymes and morphological liver changes) in treated rats, although the technical grade appeared to be slightly more potent than pure hexachlorobenzene in its effects on the pulmonary endothelium. The impurities did not appear to have a synergistic effect.

Kluwe et al. (1982) reported that pretreatment of male Sprague-Dawley rats with hexachlorobenzene resulted in increased CCl_4 toxicity. The rats received seven doses of hexachlorobenzene at 30 mg/kg once every 72 hours followed by an i.p. injection of CCl_4 at 0.0, 0.03, 0.05, 0.25, 1.0 or 2.0 mℓ/kg 24 hours after the last hexachlorobenzene treatment. Hexachlorobenzene pretreatment increased the CCl_4-induced acute growth retardation, renal tubular functional impairment, hepatocellular necrosis and further reduced the survival of the animals. Variable results were reported in a study on the effect of hexachlorobenzene pretreatment of male albino

Sprague-Dawley rats on the *in vivo* biotransformation, residue deposition, and elimination of ^{14}C-aldrin, 1-naphthol, DDT, hexachlorobenzene or mirex (Clark et al., 1981a). There was no evidence of qualitative changes in the biotransformation of any test compound that could be attributed to hexachlorobenzene pretreatment. Analysis of residue deposition gave mixed results: less ^{14}C residues were found in rats fed diets containing hexachlorobenzene and then treated with ^{14}C-aldrin, more ^{14}C residues were found after ^{14}C-DDT or ^{14}C-mirex treatment, and no difference was evident after ^{14}C-hexachlorobenzene or ^{14}C-1-naphthol treatment. Hexachlorobenzene also potentiates the effects of stress on male Sprague-Dawley rats (Clark et al., 1981). Rats fed 250 ppm hexachlorobenzene resulted in an increased severe loss of body weight when placed into crowded cages and compared to the weight loss of crowded control rats. Crowded rats fed hexachlorobenzene had higher tissue residues of hexachlorobenzene and higher mortality than the non-crowded hexachlorobenzene treated rats or the control rats.

12.5. SUMMARY

The acute oral toxicity of hexachlorobenzene has been found to be low, with LD_{50} values ranging from 1700-10,000 mg/kg. Subchronic oral toxicity studies with a number of mammalian species indicated a significant increase in liver and kidney weights in hexachlorobenzene-treated animals. Studies have shown increases in other organs as well. The livers from hexachlorobenzene-exposed animals have shown histologic changes such as irregular shaped and moderately enlarged liver mitochondria and increases in the size of the centrilobular hepatocytes. Chronic oral toxicity studies revealed the same type of effects seen in the subchronic studies plus hexachlorobenzene-associated life-shortening and various hepatic and renal pathologies.

These subchronic and chronic effects were usually dose-related. Other effects included multiple alopecia and scabbing, together with neurologic effects in rats, mice and dogs. A dose-related histopathologic change in the ovaries of monkeys has also been reported.

Increased porphyrin levels in the liver and in urine have been reported for all species studied except the dog, which does not exhibit increased porphyrin levels. Hexachlorobenzene was found to cause the accumulation of ß-H-steroids which induce porphyrin biosynthesis and to inhibit uroporphyrinogen decarboxylases. The inhibition of uroporphyrinogen decarboxylases appears to be due to pentachlorophenol, a hexachlorobenzene metabolite. Indications are that females are more susceptible to hexachlorobenzene-induced porphyria than are males, which may be related to the female estrogen levels and greater hexachlorobenzene metabolism. Hexachlorobenzene was reported to produce a mixed-type induction of cytochromes resembling that produced by a combination of phenobarbital (P-450) and 3,4-benzpyrene (P-448). In addition, the activities of several hepatic microsomal enzymes were found to be induced by hexachlorobenzene.

Hexachlorobenzene did not induce dominant lethal mutations in two studies but was reported to be mutagenic in a yeast, _S. cerevisiae_, assay at a concentration of 100 ppm. Hexachlorobenzene possessed no detectable levels of mutagenic activity in the _Salmonella_ histidine reversion assay. The chronic toxicity studies provide sufficient evidence of the carcinogenicity of hexachlorobenzene in animals since there was an increased incidence of malignant tumors of the liver in two species, haemangioendothelioma in hamsters and hepatocellular carcinoma in rats as well as confirmed reports of hepatoma in both of these species. Hexachlorobenzene was found to cause teratogenic effects in fetal mice whose mothers were ingesting 100 mg/kg/day of hexachlorobenzene during days 7-16 of gestation. Certain chemicals were found to alter the toxicity of hexachlorobenzene in mammals, whereas hexachlorobenzene pretreatment was reported to increase CCl_4 toxicity and alter the immune responses of treated animals.

13. OVERVIEW OF EFFECTS OF MAJOR CONCERN

A primary factor in identifying the major effects of concern resulting from exposure to the chlorinated benzenes is the extent and adequacy of the available studies on mammalian and human toxicology. As indicated in the section on research needs (see Section 2.3.), several areas related to the toxicity of these chemicals have not been investigated. Except for hexachlorobenzene, few studies have been performed on the carcinogenic, reproductive and teratogenic toxicity of chlorinated benzenes. However, data are available on the subchronic toxic effects produced by the oral and inhalation routes of exposure for most of the chlorinated benzenes in several species. Studies that provide adequate data on the consequences of chronic exposure or reproductive and teratologic effects of particular chlorinated benzenes do exist, but are more limited in number. The absence of discussion or presentation of data on a particular chlorinated benzene should not be equated with an absence of effects or diminished need for concern; more likely, it reflects a lack of adequate investigation.

13.1. PRINCIPAL EFFECTS AND TARGET ORGANS

The data available for identifying the principal effects and sites of toxicity for the chlorinated benzenes are derived mainly from studies of subchronic toxicity, reproductive and teratogenic effects, and reports of effects on humans accidentally or occupationally exposed to chlorinated benzenes. In general, the main sites affected by short-term, high-level exposures are the hepatic, renal and nervous systems. Inhalation and oral toxicity studies in several species indicate that chlorinated benzenes are capable of inducing hepatic and renal degeneration and necrosis, disrupting porphyrin metabolism, and depressing the short-term functioning of the nervous system. Levels of exposure below those causing hepatic and renal toxicity for some of the chlorinated benzenes have adverse effects on the long-term functioning of the nervous system and on the hematopoietic system. In several studies, the administration of two of the chlorinated benzenes, penta- and hexachlorobenzene, during gestation in rats resulted in increased fetotoxicity, postnatal mortality and incidence of fetal skeletal malformations. Studies in rodents have also shown hexachlorobenzene to be a carcinogen.

Monochlorobenzene, when administered to rats, rabbits and dogs at moderate to high doses by inhalation or oral routes caused hepatic and renal toxicity manifested by increased liver and kidney weights, histopathologic changes, elevated serum enzymes, and liver and kidney necrosis (Monsanto 1967a,b; Irish, 1963; Khanin, 1969; Dilley, 1977). At high doses, dogs developed depression of bone marrow activity (Monsanto 1967a, 1978). Continuous exposure by inhalation at low doses disturbed the proper chronaxy correlation of the muscle antagonists and increased blood cholinesterase in rats (Tarkhova, 1965). Humans exposed occupationally to monochlorobenzene intermittently for up to 2 years displayed signs of neurotoxicity including numbness, cyanosis, hyperesthesia and muscle spasms (Rozenbaum, 1947).

Subchronic administration of dichlorobenzenes by inhalation to rats, rabbits and guinea pigs caused liver and kidney toxicity and pulmonary congestion (Hollingsworth et al., 1956). Oral administration produced hepatic porphyria, pathologic changes in the kidneys and liver, and inhibition of erythropoiesis and bone marrow activity (Rimington and Ziegler, 1963; Hollingsworth et al., 1956; Varashavskaya, 1976a,b). Chronic administration of 1,2-dichlorobenzene by gavage to rats and mice at less than maximum tolerated doses did not produce statistically significant changes in tumor incidences (NTP, 1982). Case studies of human exposures report a range of effects including liver necrosis, depression of erythropoiesis and leukemia. A study of 26 persons exposed to 1,2-dichlorobenzene for 4 work days reported increased chromosomal aberrations in peripheral leukocytes (Zapata-Gayon et al., 1982).

Studies of the subchronic inhalation toxicity of 1,2,4-trichlorobenzene have identified hepatic porphyria and cellular degeneration as effects in rats but not in rabbits or monkeys (Coate et al., 1977; Watanabe et al., 1978). Porphyria was also induced in rats after the dietary administration of high doses of 1,2,3- or 1,2,4-trichlorobenzene for 7 days (Rimington and Ziegler, 1963). Three studies using dermal applications of 1,2,4-trichlorobenzene or a mixture of 1,2,4- and 1,2,3-trichlorobenzene to rabbits and guinea pigs reported skin irritation at doses as low as 30 mg/kg/day and some systemic toxicity at higher doses (Brown et al., 1969; Powers et al., 1975; Rao et al., 1982). In a reproductive study in rats, 25, 100 or 400 ppm of 1,2,3-trichlorobenzene administered to the parental animals in their drinking water produced no reproductive, hematologic or neurologic effects (Robinson et al., 1981). Adrenal enlargement occurred in both the parents and offspring at the highest dose level. In a 2-year mouse skin painting study (Yamamoto et al., 1957) a slight increase in tumors of all sites was reported, but no conclusions can be drawn about carcinogenicity because of the lack of details in the English translation of the text.

More limited data were available on the toxicity of the tetrachlorobenzenes. A single oral subchronic study with 1,2,4,5-tetrachlorobenzene in rabbits indicated effects on blood chemistry and hematology at low doses (Fomenko, 1965); a chronic study with the same isomers in dogs suggested adverse effects on liver metabolism (Braun et al., 1978). In a study of workers exposed to 1,2,4,5-tetrachlorobenzene found an increased incidence of chromosomal abnormalities (decreased chromosome number per cell, polyploidy during mitosis, and chromosomal malformations) in the leukocytes of the workers (Kiraly et al., 1979).

Data on the toxicity of pentachlorobenzene were also limited. High levels in the diets of rats caused increased excretion of porphyrins (Goerz et al., 1978) and induced histopathologic changes in the kidneys and liver (Linder et al., 1980). Studies of the reproductive and teratologic effects of pentachlorobenzene in rats indicated that the chemical increased fetal deaths, reduced postnatal survival of pups and increased the incidence of extra ribs and sternal defects (Linder et al., 1980; Khera and Villeneuve, 1975). Teratogenic effects were not seen in mice (Courtney et al., 1979).

The toxicity of long-term dietary exposure of humans to hexachlorobenzene was demonstrated by the epidemic of porphyria cutanea tarda (PCT) in Turkish citizens who accidentally consumed bread made from grain treated with hexachlorobenzene (Cam, 1963; Peters et al., 1966; Peters et al., 1982). In addition to the PCT-associated symptoms of skin lesions, hypertrichosis, and hyperpigmentation, the exposure caused neurotoxicity and liver damage. Follow-up studies reported PCT symptoms, reduced growth, and arthritic changes in the appendages of children who were directly or indirectly (i.e., through breast milk) exposed. Studies in rats have demonstrated hexachlorobenzenes ability to increase the incidence of stillbirths, decrease fetal growth and decrease postnatal survival (Grant et al., 1977; Khera, 1974). A study in rats reported that administration of hexachlorobenzene during gestation increased significantly the number of fetuses with extra ribs. A study in mice found that hexachlorobenzene given on days 7-16 of gestation resulted in an increased incidence of fetal abnormalities when compared to controls (Courtney et al., 1976). Hexachlorobenzene has been shown to produce tumors in animals. Lifetime dietary administration of hexachlorobenzene to hamsters, rats and mice increased the incidence of thyroid tumors in hamsters (Cabral et al., 1977), liver tumors in hamsters (Cabral et al., 1977), mice (Cabral et al., 1979) and rats (Smith and Cabral, 1980; Lambrecht, 1983; Arnold, 1984), kidney tumors in rats (Lambrecht, 1983) and adrenal tumors in rats (Arnold, 1983; Peters et al., 1983).

13.2. ANIMAL TOXICITY STUDIES USEFUL FOR HEALTH ASSESSMENT AND ESTIMATED TOXICITY THRESHOLDS

13.2.1. Animal Toxicity Studies. The studies useful for health assessment determinations of each of the chlorinated benzenes is presented in the respective dose/effect Tables 13-1 through 13-7, extracted from the Mammalian Toxicity Sections of Chapters 7-12 of this document. These tables should provide assistance in selecting the most useful and appropriate studies for health assessment determinations.

Tables 13-8 through 13-12 attempt to compare a variety of toxic responses to chlorinated benzenes in rats, mice, rabbits, dogs and monkeys. The recorded values reflect the lowest dosage reported for each listed effect category for each species, taken from the subchronic, chronic, carcinogenicity, reproductive and teratogenicity studies reported in Chapters 7-12 of this document. It should be noted that there is the potential for similar responses in each species to occur at lower dose levels than reported and that a blank entry does not necessarily mean that the effect does not occur in that species induced by the particular chlorinated benzene. This is probably indicative of the fact that lower dose levels may not have been tested and/or that particular effect may not have been specifically looked for by research investigators. In attempting to use these tables to determine fine-line conclusions/interpretations about chlorinated benzenes structure activity relationships, further complications arise

TABLE 13-1

Summary of Subchronic Toxicity Studies on Monochlorobenzene[a]

Species	Route	Dose	Duration (days)	Effects	Reference
Dog (beagle)	inhalation[b]	0.75 mg/ℓ, 6 hrs/day, 5 days/week (162 ppm)	62 exposures over 90 days	None	Monsanto, 1978
		1.50 mg/ℓ, 6 hrs/day, 5 days/week (424 ppm)	62 exposures over 90 days	Weight loss; conjunctivitis; moribund at 31 days	
		2.00 mg/ℓ, 6 hrs/day, 5 days/week	62 exposures over 90 days	Weight loss; hypoactivity and conjunctivitis; vacuolated hepatocytes; cytoplasmic vacuolation of renal collecting tubules; bilateral atrophy of seminiferous tubules; lower total leukocyte counts, elevated SAP, SGOT, SGPT; aplastic bone marrow; mortality in 5/8 dogs after 25-29 days	
Rat	inhalation	0.75, 1.50 or 2 mg/ℓ 6 hrs/day, 5 days/week	62 exposures over 90 days	None	Monsanto, 1978
Rat	inhalation	0.1 or 1.0 mg/m^3 (continuous)	72-80	Liver necrosis and regeneration; kidney hyperplasia; encephalopathy; pneumonia	Khanin, 1977
Rat	inhalation	0.1 mg/m^3 (continuous)	60	None	Tarkhova, 1965
		1.0 mg/m^3 (continuous)	60	Inhibited chronaxia of antagonistic muscles at 39 days; increased blood cholinesterase	
Rat	inhalation	0.1, 1.25 or 1.5 mg/ℓ	49-98	Chronaximetric inhibition	Pislaru, 1960
Rat	inhalation	0.1 mg/ℓ, 3 hr/day (alternate days)	37 weeks	Inhibition of extensor tibialis 7-14 weeks; normal by 20 weeks	Gabor and Raucher, 1960
Rat	inhalation	75 and 250 ppm, 7 hrs/day 5 days/week	120 exposures	Focal lesions of adrenal cortex; lesions in tubules of kidneys; congestion of liver and kidneys; decreased SGOT	Dilley, 1977
Rabbit	inhalation	75 and 250 ppm, 7 hrs/day, 5 days/week	120 exposures	Decreased SGOT after 24 weeks of exposure	Dilley, 1977

TABLE 13-1 (cont.)

Species	Route	Dose	Duration (days)	Effects	Reference
Mouse	oral (gavage)	60 mg/kg/day, 5 days/week	13 weeks	one male with hepatic necrosis	NTP, 1983
		125 mg/kg/day, 5 days/week	13 weeks	Increased liver weights in males one male with hepatic necrosis	
		250 mg/kg/day, 5 days/week	13 weeks	>50% reduction in weight gain, increased excretion of coproporphyrins in females, increased liver weights, lesions of the liver, kidney, bone marrow, spleen and thymus	
		500 mg/kg day, 5 days/week	13 weeks	100% lethal to males within 1 week, reduced body weight gains, polyuria in females, increased liver weights, lesions of the liver, kidney, bone marrow, spleen and thymus.	
		750 mg/kg/day, 5 days/week	10 weeks	100% lethal to male mice within 1 week and to female mice within 10 weeks, lesions of the liver, kidney, bone marrow, spleen and thymus at death	
Rat	Oral (gavage)	60 mg/kg/day, 5 days/week	13 weeks	None	NTP, 1983
		125 mg/kg/day, 5 days/week	13 weeks	None	
		250 mg/kg/day, 5 days/week	13 weeks	Minimal centrolobular hepatocellular necrosis	
		500 mg/kg/day, 5 days/week	13 weeks	Decreased body weights gain, increased GGTP and alkaline phosphatase in females, increased excretion of porphyrins, centrolobular hepatocellular necrosis, nephropathy in males, myeloid depletion of bone marrow.	
		750 mg/kg day, 5 days/week	13 weeks	Decreased body weight gain and survival of animals, hematologic effects, increased GGTP and alkaline phosphatase in females, polyuria in males, increased excretion of porphyrins, centrolobular hepatocellular necrosis, nephropathy, lymphoid depletion of thymus and spleen, myeloid depletion of bone marrow.	

TABLE 13-1 (cont.)

Species	Route	Dose	Duration (days)	Effects	Reference
Dog	oral (capsule)	27.3 mg/kg/day	90	None	Monsanto, 1967a
		54.6 mg/kg/day	90	Diarrhea and vomiting; conjunctivitis	
		272.5 mg/kg/day	90	4/8 died in 3-5 weeks; increased immature leukocytes; elevated SGOT and SAP, bilirubin and cholesterol; low blood sugar; histopathologic changes in liver, kidneys, spleen	
Rat	oral (diet)	12.5 or 50 mg/kg/day	93-99	None	Monsanto, 1967b
		100 mg/kg/day	93-99	Increased liver and kidney weights	
		250 mg/kg/day	93-99	Increased liver and kidney weights; retarded growth in males	
Rat	oral (diet)	14.4 mg/kg/day	192	None	Irish, 1963
		144 and 288 mg/kg/day	192	Increased liver and kidney weights; increased salivation and hair loss	

[a] Source: Updated from U.S. EPA, 1980a

[b] 1 ppm ~4.60 mg/m^3, 1 mg/ℓ ~219 ppm (Irish, 1963)

TABLE 13-2

Subchronic Toxicity of 1,2-Dichlorobenzene*

Route	Concentration or Dose	Regimen	Subject	Effect	Reference
Inhalation	560 mg/m^3	7 hours/day, 5 days/week, 6-7 months	rat, guinea pig, rabbit, monkey	No effect on several parameters except decreased spleen weights in male guinea pigs	Hollingsworth et al., 1958
	290 mg/m^3	7 hours/day, 5 days/week 6.5 months	rat, guinea pig	No effect on several parameters	Hollingsworth et al., 1958
	455 mg/m^3	daily up to 15 days	rat	Hepatic porphyria	Rimington and Ziegler, 1963
Oral	376 mg/kg (tube)	5 days/week, 138 doses	rat	Liver, kidney weight increase; cloudy swelling in liver.	Hollingsworth et al., 1958
	188 mg/kg (tube)	5 days/week, 138 doses	rat	Increase in liver and kidney weight	Hollingsworth et al., 1958
	18.8 mg/kg (tube)	5 days/week, 138 doses	rat	No effects noted	Hollingsworth et al., 1958
	0.01-0.1 mg/kg/day	5 months	rat	Hematopoietic system; altered conditioned reflexes; increased prothromb time and altered enzyme activities	Varshavskaya, 1967a
	500 mg/kg	5 days/week, 13 weeks	rat	Increased liver weights; polyuria in males; increased urinary porphyrins; hepatic necrosis and degeneration; renal tubular degeneration; thymic lymphoid depletion; and hematologic and clinical changes	NTP, 1982
	250 mg/kg	5 days/week, 13 weeks	rat	Increased liver weights; hematologic and clinical changes; hepatic necrosis	NTP, 1982
	125 mg/kg	5 days/week, 13 weeks	rat	Increased liver weights; hematologic and clinical changes; some hepatic necrosis	NTP, 1982
	60 mg/kg	5 days/week, 13 weeks	rat	Hematologic and clinical changes	NTP, 1982
	30 mg/kg	5 days/week, 13 weeks	rat	Hematologic and clinical changes	NTP, 1982

TABLE 13-2 (cont.)

Route	Concentration or Dose	Regimen	Subject	Effect	Reference
Oral (cont.)	500 mg/kg	5 days/week, 13 weeks	mouse	Increased mortality; increased liver weights; increased urinary and liver porphyrins; hepatic necrosis and degeneration; heart and skeletal muscle lesions; lymphoid depletion of thymus and spleen	NTP, 1982
	250 mg/kg	5 days/week, 13 weeks	mouse	Hepatic necrosis and degeneration in males; no effects in females	NTP, 1982
	30, 60, 125 mg/kg	5 days/week, 13 weeks	mouse	No effects	NTP, 1982
Subcutaneous	unspecified	repeated	rabbit	Blood dyscrasias, (agranulocytosis)	Ware and West, 1977

*Source: Modifed from U.S. EPA, 1980c

TABLE 13-3

Subchronic Toxicity of 1,4-Dichlorobenzene*

Route	Concentration or Dose	Regimen	Subject	Effect	Reference
Inhalation	10^5 mg/m³	0.5 hours/day, 5-9 days	rabbit	Granulocytopenia; irritation; CNS and lung toxicity; death (12/18)	Zupko and Edwards, 1949
	4800 mg/m³	8 hours/day, 5 days/week, up to 69 exposures	rat, guinea pig, rabbit	Severe irritation; CNS depression and collapse; liver, kidney, lung pathology; deaths	Hollingsworth et al., 1956
	4600-4800 mg/m³	8 hours/day, 5 days/week,	rabbit	Tremors, weakness, nystagmus; some deaths	Pike, 1944
	2050 mg/m³	7 hours/day, 5 days/week, 6 months	rat, guinea pig	Growth depression, increased liver, kidney weight; liver pathology (necrosis, fatty degeneration, swelling, fibrosis)	Hollingsworth et al., 1956
	1040 mg/m³	7 hours/day, 5 days/week, 16 days	rat, guinea pig	Increased liver, kidney weight (rat); lung, liver pathology	Hollingsworth et al., 1956
	950 mg/m³	7 hours/day, 5 days/week, 157-219 days	rat, guinea pig, rabbit, mouse, monkey	Growth depression (guinea pig); increased liver, kidney weight; histological liver changes (cloudy swelling, granular degeneration) in rats, no adverse effects reported in rabbit, mouse or monkey	Hollingsworth et al., 1956
	900 mg/m³	8 hours/day, 2 weeks	mouse	Respiratory excitation; liver pathology, deaths; at serum concentration of 39 mg/ℓ	Irie et al., 1973
	580 mg/m³	7 hours/day, 5 days/week, 6-7 months	rat, guinea pig, mice, rabbit, monkey	No adverse effects on several parameters	Hollingsworth et al., 1956
Oral	1000 mg/kg per dose (tube)	92 doses in 219 days	rabbit	CNS depression; weight loss; liver degeneration and necrosis; deaths	Hollingsworth et al., 1956
	770 mg/kg/day	up to 5 days	rat	Hepatic porphyria	Rimington and Ziegler, 1963
	500 mg/kg/day (tube)	5 days/week, 20 doses	rat	Hepatic centrolobular necrosis; cloudy swelling, renal tubular epithelium, and casts	Hollingsworth et al., 1956

TABLE 13-3 (cont.)

Route	Concentration or Dose	Regimen	Subject	Effect	Reference
Oral (cont.)	5000 mg/kg diet	up to 35 days	Peking duck	Death in 3/10. Retarded growth	Hollingsworth et al., 1956
	500 mg/kg/day (tube)	5 days/week, 263 doses in 367 days	rabbit	CNS depression; weight loss; liver pathology	Hollingsworth et al., 1956
	376 mg/kg/day	5 days/week, 138 doses in 192 days	rat	Increased liver and kidney weight; liver cirrhosis and focal necrosis	Hollingsworth et al., 1956
	250 mg/kg/day	3 days	rat	Induced liver metabolism enzyme system	Ariyoshi et al., 1975a,b
	188 mg/kg/day	5 days/week, 138 doses in 192 days	rat	Increased liver and kidney weight	Hollingsworth et al., 1956
	20-40 mg/kg/day	2 weeks	rat	Induced liver metabolism enzyme system	Carlson and Tardiff, 1976
	18.8 mg/kg/day	5 days/week, 138 doses in 192 days	rat	No adverse effects detected	Hollingsworth et al., 1956

*Source: U.S. EPA, 1980c

TABLE 13-4

Summary of Subchronic and Chronic Toxicity Studies on Trichlorobenzenes

Species	Route	Dose	Duration	Effects	Reference
Rat	inhalation	74.2, 742 or 7423 mg/m³ of 1,3,5-TCB	6 hr/day, 5 day/wk for up to 13 wk	No hepatotoxicity; three high-dose rats had squamous metaplasia and focal hyperplasia of respiratory epithelium, believed to be reversible	Sasmora and Palmer, 1981
Rats, rabbits, two dogs	inhalation	223 or 742 mg/m³ of 1,2,4-TCB	7 hr/day, 5 day/wk; total of 30 exposures in 44 days	Increase in urinary excretion of porphyria in exposed rats; increase in liver weights in high-dose rats and dogs; increased kidney weights in high-dose rats	Kociba et al., 1981
Rat	inhalation	22.3 or 74.2 mg/m³ of 1,2,4-TCB	6 hr/day, 5 day/wk, 3 mo	Increase in urinary porphyrin excretion in high-dose rats; no effects in 22.3 mg/m³ group	Watanabe et al., 1978
Rat	inhalation	186, 371 or 742 mg/m³ of 1,2,4-TCB	7 hr/day, 5 day/wk, 26 wk	Enlarged hepatocytes and nondose-dependent hepatocytes vacuolization, liver granulance, biliary hyperplasia and kidney hyaline degeneration at 4 and 13 wk; no histopathology evident at 26 wk	Coate et al., 1977
Rabbits, monkeys	inhalation	186, 371 or 742 mg/m³ of 1,2,4-TCB	7 hr/day, 5 day/wk, 26 wk	No treatment related changes at 26 wk	Coate et al., 1977
Monkey	oral	1, 5, 25, 90, 125 or 173.6 mg/kg/day of 1,2,4-TCB	30 days	≤25 mg/kg/day - no effects observed; ≥90 mg/kg/day - observed toxicity and death	Smith et al., 1978
Rat	oral	50, 100 or 200 mg/kg/day of 1,2,4-TCB	30, 60, 90 or 120 days	Increases in liver weights, liver porphyrins and urine porphyrins, dose and time related	Carlson, 1977b
Rat	oral	10, 20 or 40 mg/kg/day of 1,2,4-TCB	90 days	Increase in liver-to-body weight ratio in high-dose group; changes in enzyme activation at all doses	Carlson and Tardiff, 1976
Mouse	oral	600 ppm diet (0.078 mg/kg/day) of 1,2,4-TCB	6 mo	No effects	Goto et al., 1972

TABLE 13-4 (cont.)

Species	Route	Dose	Duration	Effects	Reference
Guinea pig	dermal	0.5 mℓ/day of 1,2,4-TCB	5 day/wk, 3 wk	Death following extensor convulsion; livers showed necrotic foci	Brown et al., 1969
Mouse	dermal	0.003 mℓ/painting of 30 and 60% solution in acetone of 1,2,4-TCB	2 times/wk, 2 yr	Painting induced excitability, panting and epidermal thickening, inflammation and keratinization; increased organ weights and mortality	Yamamoto et al., 1957
Rats	oral (drinking water)	25, 100 or 400 mg/ℓ of 1,2,4-TCB	F_0 to F_2 generations	Enlarged adrenals in F_0 and F_1 generations	Robinson et al., 1981
Rabbits	dermal	30, 150 or 450 mg/kg/day of 1,2,3-TCB	5 day/wk, 4 wk	Dose-related skin irritation; increase in urinary coproporphyrin in high-dose males and slight pallor of liver in males and females	Rao et al., 1982

1,2,3-TCB = 1,2,3-trichlorobenzene; 1,2,4-TCB = 1,2,4-trichlorobenzene; 1,3,5-TCB = 1,3,5-trichlorobenzene

TABLE 13-5

Summary of Toxicity Studies on Tetrachlorobenzenes

Species	Route	Dose	Duration	Effects	Reference
Rat	oral	0.5-500 mg/kg of diet 1,2,4,5-TeCB	28 or 90 days	Increased liver and kidney weights and histological changes in liver and kidneys; increases in MFO activity, serum cholesterol values	Villeneuve et al., 1983
Rat	oral	0.001, 0.005, 0.05 mg/kg/day 1,2,4,5-TeCB	8 mo	No effects observed in 0.001 mg/kg/day dose group; 0.005 and 0.05 mg/kg/day doses caused disruption in conditioned reflexes, increases in liver weight coefficients and decrease in serum SH groups	Fomenko, 1965
Rabbit	oral	0.001, 0.005, 0.05 mg/kg/day 1,2,4,5-TeCB	8 mo	No effect observed in 0.001 mg/kg/day dose group; 0.05 mg/kg dose caused disorder of liver glycogen formation, altered serum SH group levels, increase in blood hemoglobin and peripheral reticulocyte levels	Fomenko, 1965
Rat	oral	75 mg/kg/day 1,2,4,5,-TeCB	2 mo	Altered biochemical parameters indicating changes in hepatic and hematopoitic homeostasis	Fomenko, 1965
Dog	oral	5 mg/kg/day 1,2,4,5-TeCB	2 yr exposure, 22 mo recovery	No controls used; elevated SAP and total bilirubin, returned to normal range 3 mo after exposures ended	Braun et al., 1978
Pregnant rats	oral	50, 100, 200 mg/kg/day 1,2,4,5-TeCB	days 6-15 of gestation	High-dose lethal to 9/10 of treated dams; organ weight changes, elevated serum cholesterol and liver metabolism enzymes, no indication of those changes were dose-related	Ruddick et al., 1981
Pregnant rats	oral	50, 100, 200 mg/kg/day 1,2,3,4-TeCB	days 6-15 of gestation	Induced maternal toxicity and increased lethality of pups at 200 mg/kg/day	Ruddick et al., 1981
Pregnant rats	oral	50, 100, 200 mg/kg/day 1,2,3,5-TeCB	days 6-15 of gestation	Increased lethality in 200 mg/kg/day group pups; one pup malformed and minor chondrogenic delay in other pups	Ruddick et al., 1981

1,2,4,5-TeCB = 1,2,4,5-tetrachlorobenzene
1,2,3,4-TeCB = 1,2,3,4-tetrachlorobenzene
1,2,3,5-TeCB = 1,2,3,5-tetrachlorobenzene

TABLE 13-6

Summary of Subchronic, Reproductive and Teratogenic Toxicity Studies on Pentachlorobenzene

Species	Route	Dose	Duration	Effects	Reference
Rat (female)	oral (diet)	125, 250, 500 or 1000 mg/kg in diet	180 days	Changes in hematologic parameters in high-dose group; increase in liver weights, hepatic hypertrophy and vacuolization in 500 and 1000 mg/kg groups; increased kidney weight in high-dose group	Linder et al., 1980
Rat (male)	oral (diet)	125 or 1000 mg/kg in diet	100 days	High-dose group induced changes in hematologic parameters; hepatic and renal histology and increase in liver, kidney and adrenal weights	Linder et al., 1980
Rat (offspring)	oral (diet)	125, 250, 500 or 1000 mg/kg in mothers diet	gestation and during suckling	Offspring treated with ≥250 mg/kg/diet were adversely affected (reduced survival, body weights and increased liver weights, hepatocellular enlargement)	Linder et al., 1980
Mice	oral	50 or 100 mg/kg/gavage	days 6-15 of gestation	Increase in liver weights of dams; no adverse effects on total development or survival	Courtney et al., 1979
Rat	oral	50, 100 or 200 mg/kg/gavage	days 6-15 of gestation	No observed toxicity in adult rats; increased total deaths at all doses, but not in dose-related manner; extra ribs in exposed fetuses and sternal defects in 200 mg/kg group	Khera and Villeneuve, 1975

TABLE 13-7

Summary of Toxicity Studies on Hexachlorobenzene

Species	Route	Dose	Duration	Effects	Reference
Rat (females)	oral	100 mg/kg every other day	up to 43 days	Suggested covalent binding of hexachlorobenzene metabolites to cytosolic proteins	Koss et al., 1980a
Rat	oral (diet)	0.5 mg/kg/day	15 weeks exposed and held to 48 weeks	Transient increases in liver porphyrin levels in females after termination of exposure	Kuiper-Goodman et al., 1977
		2.0 mg/kg/day	15 weeks exposed and held to 48 weeks	Increases in liver porphyrin levels in females after termination of exposure, increased size of centrilobular hepatocytes	
		8.0 mg/kg/day	15 weeks exposed and held to 48 weeks	Increased liver weights, increased liver, kidney and spleen porphyrin levels in females (porphyria), centrilobular liver lesions especially in females at 48 weeks	
		32.0 mg/kg/day	15 weeks exposed and held to 48 weeks	Increased mortality in females, intension tremors in males and females and ataxia in a few females, increased liver, kidney and spleen weights, increased liver, kidney and spleen porphyrin levels in females (porphyria), centrilobular liver lesions and splenomegaly	
Rat (females)	oral (gavage)	50 mg/kg every other day	15 weeks	Increased liver, kidney, spleen and adrenal weights, porphyria (increased liver porphyrin levels and increased excretion of porphyrins and precursors), tremors, hair loss and skin lesions	Koss et al., 1978b
Rats (females)	oral (gavage)	0.5 mg/kg twice weekly	29 weeks	Increase in relative liver weight	Böger et al., 1979
		2.0 mg/kg twice weekly	29 weeks	Increase in relative liver weight, moderately enlarged hepatocytes	
		8.0 mg/kg twice weekly	29 weeks	Porphyria, markedly enlarged hepatocytes, increase in relative liver weight	
		32.0 mg/kg twice weekly	29 weeks	Porphyria, markedly enlarged hepatocytes, increase in liver weights	
Rat (females)	oral (diet)	100 mg/kg diet	98 days	Porphyria (increased liver lobe porphyrins), decreased activity of uroporphyrinogen decarboxylase	Smith et al., 1980

TABLE 13-7 (cont.)

Species	Route	Dose	Duration	Effects	Reference
Rat	oral (diet and nursing)	50 mg/kg diet	gestation until 5 weeks of age	Depressed resistance to L. monocytogenes and T. spiralis, enhanced thymus-dependent antibody response	Vos et al., 1979b
		150 mg/kg diet	gestation until 5 weeks of age	Increased serum IgM and IgG, depressed resistance to L. monocytogenes and T. spiralis, enhanced thymus-dependent antibody response, increased liver and adrenal weights	
Rat	oral (diet)	500, 1000 or 2000 mg/kg diet	3 weeks	Dose-related increases in relative spleen, lymph nodes, liver, adrenals, thyroid, testes and kidney weights, dose-related increase in serum IgM levels, no change in serum IgG levels, dose-related pathological changes in liver, lymph nodes and spleen	Vos et al., 1979a
Rat	oral (diet)	2000 mg/kg diet	10 weeks	Porphyria found microscopically at 5 weeks and grossly at 10 weeks using fluorescence	Gralla et al., 1977
Rat (male)	oral (diet)	2000 mg/kg diet	100 days	Elevated hepatic enzymes by 1 week and increased urinary porphyrin and ALA levels (porphyria) as early as 40 days	Lissner et al., 1975
Rat (female)	oral (diet)	3000 mg/kg diet	11 weeks	Decreased uroporphyrinogen decarboxylase activity and porphyria after 4 weeks	Elder et al., 1976
Rat (female)	oral (gavage)	50, 100 or 200 mg/kg	120 days	Dose- and time-dependent increase in liver and urine porphyrins (porphyria)	Carlson, 1977b
Rat	oral (gavage)	14 mg/kg every other day	103 days	Porphyria in treated females, susceptibility of females to porphyria may be related to estrogen levels	Rizzardini and Smith, 1982
Rat (females)	oral (gavage)	100 mg/kg every other day	6 weeks exposed and held for additional 18 months	Porphyria (liver uroporphyrin levels peaked 7 months postexposure and levels had not returned to normal by 18 months), decreased liver protoporphyrin and coproporphyrin levels, inhibition of uroporphyrinogen decarboxylase activity until 18 months postexposure	Koss et al., 1983
Rat (females)	oral (diet)	6-8 mg/kg/day	75-90 weeks	Decline in body weights, porphyria, enlarged livers and liver tumors	Smith and Cabral, 1980
Rat	oral (diet)	75 mg/kg diet (4-5 mg/kg/day) 150 mg/kg diet (8-9.5 mg/kg/day)	up to 2 years	Porphyria, time-related appearance of severe hepatic and renal pathologies, after 1 year increases in hepatomas, hepatocarcinomas, bile duct adenomas, renal adenomas and renal carcinomas	Lambrecht et al., 1983a,b

TABLE 13-7 (cont.)

Species	Route	Dose	Duration	Effects	Reference
Rat	oral (diet)	0.32, 1.6, 8.0 or 40 mg/kg diet	~130 days	Hematological changes at all dose levels in males, increases in liver and heart weights in males at 8.0 and 40 ppm diets, no treatment-related effects observed in bred females	Arnold et al., 1983
	oral (diet and nursing)	0.32 or 1.6 mg/kg diet	gestation through lifetime (130 weeks)	Glycogen depletion in 1.6 mg/kg males; no effects reported at 0.32 mg/kg	
		8.0 mg/kg diet	gestation through lifetime (130 weeks)	Increase in liver pathologies	
		40 mg/kg diet	gestation through lifetime (130 weeks)	Increased mortality as pups, increase in liver and kidney pathologies, increase in adrenal pheochromocytomas in females and parathyroid tumors in males	
Rat	oral (diet)	10 or 20 mg/kg diet	F_0 to F_4 generations	No effects reported	Grant et al., 1977
		40 mg/kg diet	F_0 to F_4 generations	Increases in liver weights and aniline hydroxylase activity	
		80 mg/kg diet	F_0 to F_4 generations	Decreased body weights, F_3 and F_4 generations had decreased lactation index and postnatal viability and increased stillbirths	
		160 mg/kg diet	F_0 to F_4 generations	Increased mortality and decreased lactation index starting in F_1 generation	
		320 and 640 mg/kg diet	F_0 to F_4 generations	20 and 50% mortality in F_0 320 and 640 mg/kg groups, respectively, greatly reduced fertility index and litter size and increase in stillbirths, viability index zero in F_1	
Rat	oral (diet)	60, 80, 100, 120 or 140 mg/kg diet	F_0 to F_{1a} and F_{1b} generations	Increased mortality in all groups at 21 days, 21-day LD_{50} values for pups were 100 and 140 mg/kg for F_{1a} and F_{1b} generations, respectively	Kitchin et al., 1982
Rat	oral (diet)	0 or 80 mg/kg diet	gestation and nursing or cross nursed with controls	Nursing exposure produced greater effects than did gestational exposure, effects noted were: smaller brains, hearts, kidneys and spleens, increased liver weights	Mendoza et al., 1978
Rat	oral (diet)	80 mg/kg diet	2 weeks prior to mating to 35-36 days after weaning	Increased porphyrin levels and decreased liver esterase activity in dams, no changes in gestation indices or neonatal survival	Mendoza et al., 1979

TABLE 13-7 (cont.)

Species	Route	Dose	Duration	Effects	Reference
Rat	oral (gavage)	10, 20, 40, 60, 80 or 120 mg/kg	days 6-21 of gestation	Maternal toxicity (weight loss, tremors and convulsions) and reduced fetal weights at 120 and 80 mg/kg maternal doses, dose-related increase in incidence of unilateral and bilateral 14th rib, sternal defects were also noted in one experiment	Khera, 1974
Mouse	oral (diet)	2.5, 25 or 250 mg/kg diet	21 days	Dose-related increase in liver and decrease in prostate and seminal vesicle weights, dose-related alterations in testosterone metabolism, altered hepatic enzyme levels	Elissalde and Clark, 1979
Mouse (male)	oral (diet)	10 mg/kg diet (8.4 (mg/mouse/24 weeks) or 50 mg/kg diet (35.3 mg/mouse/ 24 weeks)	24 weeks	Dose-related reduction in weight gain, no tumor pathology observed	Shirai et al., 1978
Mouse (male)	oral (diet)	167 mg/kg diet	3-6 weeks	Impairment in host resistance as measured by increased sensitivity to S. typhosa and P. bergheri, and decrease in IgA levels	Loose et al., 1978a,b
Mouse	oral (diet)	6, 12, 24 and 36* mg/kg/day	101-120 weeks *(15 weeks exposed held until 120 weeks)	Reduced growth rate at all dose levels, shortened lifespan associated with tremors and convulsions in 24 and 36 mg/kg/day groups, dose-dependent increase in liver-cell tumors in the 12, 24 and 36 mg/kg/day dose groups	Cabral et al., 1979
Mouse	oral (gavage)	100 mg/kg/day to pregnant mice	days 7-16 of gestation	Increased maternal livers and decreased fetal body weights, increased incidence of abnormal fetuses per litter observed	Courtney et al., 1976
Hamster	oral (diet)	200 or 400 mg/kg diet	90 days	Precirrhotic and cirrhotic hepatic lesions, bile-duct hyperplasias and hepatomas	Lambrecht et al., 1982
Hamster	oral (diet)	4, 8 or 16 mg/kg/day	lifespan	Shortened lifespan in 16 mg/kg/day group, increase in hepatomas at all dose levels, increase in liver haemangioendothelioma in males and females and an increase in thyroid alveolar adenomas in males in 16 mg/kg/day group	Cabral et al., 1977
Cats (breeding females)	oral (diet)	3 or 8.7 mg/day/cat	142 days	Weight loss and increased disease susceptibility in bred females, dose-related decrease in litter size and survival of offspring, hepatomegaly in offspring	Hansen et al., 1979

TABLE 13-7 (cont.)

Species	Route	Dose	Duration	Effects	Reference
Minks	oral (diet)	1 or 5 mg/kg diet	during gestation until 17 weeks of age	Dose-related increase in offspring mortality, induction of hepatic MFO enzymes in exposed offspring	Rush et al., 1983
Dog (female)	oral (capsule)	50 or 150 mg/kg/day	21 days	Liver and hepatocyte enlargement, dose-induced electroencephalogram dysrhythmias	Sundlof et al., 1981
Dog	oral (capsule)	1, 10, 100 or 1000 mg/day/dog	1 year	Increase in mortality, neutrophilia, and anorexia in the 100 and 1000 mg dose groups, dose-related nodular hyperplasia of gastric lymphoid tissue in all treated animals	Gralla et al., 1977
Monkey (female)	oral (gavage)	8, 32, 64 or 128 mg/kg/day	60 days	Dose-related pathology in liver, kidney, ovaries and thymus	Iatropoulus et al., 1976
Monkey	oral (nursing)	7.51-186 ppm milk	60 days	2 of 3 infants died as a result of exposures	Bailey et al., 1980

TABLE 13-8

Comparison[a,b] of Toxic Effects of Chlorinated Benzene Isomers in Rats

Chemical		Organ/Body Weight Changes	Altered Enzyme Levels	Porphyrinogenic Effects	Neurologic Effects	Hematopoietic Effects	Renal Effects	Hepatic Effects	Adrenal Effects	Reproductive and Teratogenic Effects	Carcinogenic Effects
Mono-CB	(I)	2000 mg/m^3 (90)[d]	1.0 mg/m^3 (60)		1.0 mg/m^3 (60)		0.1 mg/m^3 (80)	0.1 mg/m^3 (80)	345 mg/m^3[c] (168)		
	(O)	100 mg/kg/day (99)	500 mg/kg/day (90)	500 mg/kg/day (90)		500 mg/kg/day (90)	500 mg/kg/day (90)	250 mg/kg/day (90)			
1,2-DCB	(I)			455 mg/m^3 (15)							
	(O)	125 mg/kg (90)[e]	0.1 mg/kg/day (150)	500 mg/kg (90)[e]	0.01 mg/kg/day (150)	0.1 mg/kg/day (150)	500 mg/kg (90)[e]	125 mg/kg (90)[e]			
1,3-DCB	(I)										
	(O)										
1,4-DCB	(I)	950 mg/m^3 (219)[c]			4800 mg/m^3 (97)[f]		4800 mg/m^3 (97)[f]	950 mg/m^3 (219)[c]			
	(O)	188 mg/kg (192)[e]	20 mg/kg/day (14)	770 mg/kg/day (5)			500 mg/kg (28)[e]	376 mg/kg (192)[e]			
1,2,3-TCB	(I)										
	(O)										
1,2,4-TCB	(I)	186 mg/m^3 (90)[c]		74.2 mg/m^3 (90)[d]			186 mg/m^3 (90)[c]	186 mg/m^3 (90)[c]			
	(O)	40 mg/kg/day (90)	10 mg/kg/day (90)	100 mg/kg/day (30)					33-56 mg/kg/day (95)[g]		
1,3,5-TCB	(I)										
	(O)										
1,2,3,4-TeCB	(I)										
	(O)					200 mg/kg/day (10)				200 mg/kg/day (10)	

TABLE 13-8 (cont.)

Chemical		Organ/Body Weight Changes	Altered Enzyme Levels	Porphyrinogenic Effects	Neurologic Effects	Hematopoietic Effects	Renal Effects	Hepatic Effects	Adrenal Effects	Reproductive and Teratogenic Effects	Carcinogenic Effects
1,2,3,5-TeCB	(I)										
	(0)									200 mg/kg/day (10)	
1,2,4,5-TeCB	(I)										
	(0)	0.005 mg/kg/day (240)	75 mg/kg/day (60)		0.005 mg/kg/day (240)	75 mg/kg/day (60)			75 mg/kg/day (60)	200 mg/kg/day (10)	
PCB	(I)										
	(0)	46 mg/kg/day (180)	25 mg/kg/day (10)			97 mg/kg/day (100)	97 mg/kg/day (100)	46 mg/kg/day (180)		16-31 mg/kg/day (100)	
HCB	(I)										
	(0)	0.3 mg/kg/day (130)	5 mg/kg/day (60)	0.05 mg/kg/day (105)	50 mg/kg (105)[h]	0.01 mg/kg/day (130)	2 mg/kg/day (910)	2 mg/kg/day (105)	2 mg/kg/day (910)	10 mg/kg/day (10)	4-5 mg/kg/day (730)

[a] All values are the lowest dose level reported for each listed effect category, from the mammalian toxicity sections of Chapters 7-12, with dosing duration listed in (days). A blank indicates that the effect has not been reported in this species for this isomer.

[b] From subchronic, chronic, reproductive and teratogenicity studies

[c] mg/m³, 7 hours/day, 5 days/week

[d] mg/m³, 6 hours/day, 5 days/week

[e] mg/kg, 5 days/week

[f] mg/m³, 8 hours/day, 5 days/week

[g] In F_0 and F_1 generations: enlarged adrenals

[h] mg/kg every other day

I = Inhalation exposure; 0 = oral exposure

Mono-CB = monochlorobenzene; DCB = dichlorobenzene; TCB = trichlorobenzene; TeCB = tetrachlorobenzene; PCB = pentachlorobenzene; HCB = hexachlorobenzene

TABLE 13-9

Comparison[a,b] of Toxic Effects of Chlorinated Benzenes in Mice

Chemical		Organ/Body Weight Changes	Altered Enzyme Levels	Porphyrinogenic Effects	Neurologic Effects	Hematopoietic Effects	Renal Effects	Hepatic Effects	Adrenal Effects	Reproductive and Teratogenic Effects	Carcinogenic Effects
Mono-CB	(I)										
	(O)	125 mg/kg/day (90)		250 mg/kg/day (90)		500 mg/kg/day (90)	250 mg/kg/ day (90)	60 mg/kg/ day (90)			
1,2-DCB	(I)										
	(O)	500 mg/kg (90)[c]		500 mg/kg (90[c]		500 mg/kg (90)[c]		250 mg/kg (90)[c]			
1,3-DCB	(I)										
	(O)										
1,4-DCB	(I)				900 mg/m³ (14)[d]			900 mg/m³ (14)[d]			
	(O)										
1,2,3-TCB	(I)										
	(O)										
1,2,4-TCB	(I)										
	(O)										
1,3,5-TCB	(I)										
	(O)										
1,2,3,4-TeCB	(I)										
	(O)										
1,2,3,5-TeCB	(I)										
	(O)										
1,2,4,5-TeCB	(I)										
	(O)										

TABLE 13-9 (cont.)

Chemical		Organ/Body Weight Changes	Altered Enzyme Levels	Porphyrinogenic Effects	Neurologic Effects	Hematopoietic Effects	Renal Effects	Hepatic Effects	Adrenal Effects	Reproductive and Teratogenic Effects	Carcinogenic Effects
PCB	(I)										
	(O)	50 mg/kg/day (10)									
HCB	(I)										
	(O)	0.01 mg/kg/day (21)	0.01 mg/kg/day (21)		24 mg/kg/day (840)			12 mg/kg/day (840)		100 mg/kg/day (10)	12 mg/kg/day (840)

[a] All values are the lowest dose level reported for each listed effect category, from the mammalian toxicity sections of Chapters 7-12, with dosing duration listed in days. A blank indicates that the effect has not been reported in this species for this isomer.

[b] From subchronic, chronic, reproductive and teratogenicity studies

[c] mg/kg, 5 days/week

[d] 8 hours/day

I = Inhalation exposure; O = oral exposure

Mono-CB = monochlorobenzene; DCB = dichlorobenzene; TCB = trichlorobenzene; TeCB = tetrachlorobenzene; PCB = pentachlorobenzene; HCB = hexachlorobenzene

TABLE 13-10

Comparison^{a,b} of Toxic Effects of Chlorinated Benzenes in Rabbits

Chemical		Organ/Body Weight Changes	Altered Enzyme Levels	Porphyrinogenic Effects	Neurologic Effects	Hematopoietic Effects	Renal Effects	Hepatic Effects	Adrenal Effects	Reproductive and Teratogenic Effects	Carcinogenic Effects
Mono-CB	(I)		345 mg/m³ (168)^{c}								
	(0)										
1,2-DCB	(I)										
	(0)										
1,3-DCB	(I)										
	(0)										
1,4-DCB	(I)				4800 mg/m³ (97)^{d}		4800 mg/m³ (97)^{d}	4800 mg/m³ (97)^{d}			
	(0)	500 mg/kg (367)^{e}			500 mg/kg (367)^{e}			500 mg/kg (367)^{e}			
1,2,3-TCB	(I)										
	(0)										
1,2,4-TCB	(I)										
	(0)										
1,3,5-TCB	(I)										
	(0)										
1,2,3,4-TeCB	(I)										
	(0)										
1,2,3,5-TeCB	(I)										
	(0)										

TABLE 13-10 (cont.)

Chemical	Organ/Body Weight Changes	Altered Enzyme Levels	Porphyrinogenic Effects	Neurologic Effects	Hematopoietic Effects	Renal Effects	Hepatic Effects	Adrenal Effects	Reproductive and Teratogenic Effects	Carcinogenic Effects
1,2,4,5-TeCB (I)										
(O)					0.05 mg/kg/day (240)					
PCB (I)										
(O)										
HCB (I)										
(O)										

[a] All values are the lowest dose level reported for each listed effect category, from the mammalian toxicity sections of Chapters 7-12, with dosing duration listed in days. A blank indicates that the effect has not been reported in this species for this isomer.

[b] From subchronic, chronic, reproductive and teratogenicity studies

[c] mg/m³, 7 hours/day, 5 days/week

[d] mg/m³, 8 hours/day, 5 days/week

[e] mg/kg, 5 days/week

I = Inhalation exposure; O = oral exposure

Mono-CB = monochlorobenzene; DCB = dichlorobenzene; TCB = trichlorobenzene; TeCB = tetrachlorobenzene; PCB = pentachlorobenzene; HCB = hexachlorobenzene

TABLE 13-11

Comparison^{a,b} of Toxic Effects of Chlorinated Benzenes in Dogs

Chemical		Organ/Body Weight Changes	Altered Enzyme Levels	Porphyrinogenic Effects	Neurologic Effects	Hematopoietic Effects	Renal Effects	Hepatic Effects	Adrenal Effects	Reproductive and Teratogenic Effects	Carcinogenic Effects
Mono-CB	(I)	1500 mg/m^3 (90)^c	2000 mg/m^3 (90)^c			2000 mg/m^3 (90)^c	2000 mg/m^3 (90)^c	2000 mg/m^3 (90)^c		2000 mg/m^3 (90)^c	
	(0)		272.5 mg/kg/day (90)			272.5 mg/kg/day (90)	272.5 mg/kg/day (90)	272.5 mg/kg/day (90)			
1,2-DCB	(I)										
	(0)										
1,3-DCB	(I)										
	(0)										
1,4-DCB	(I)										
	(0)										
1,2,3-TCB	(I)										
	(0)										
1,2,4-TCB	(I)	742 mg/m^3 (44)^d									
	(0)										
1,3,5-TCB	(I)										
	(0)										
1,2,3,4-TeCB	(I)										
	(0)										
1,2,3,5-TeCB	(I)										
	(0)										

TABLE 13-11 (cont.)

Chemical		Organ/Body Weight Changes	Altered Enzyme Levels	Porphyrinogenic Effects	Neurologic Effects	Hematopoietic Effects	Renal Effects	Hepatic Effects	Adrenal Effects	Reproductive and Teratogenic Effects	Carcinogenic Effects
1,2,4,5-TeCB	(I)										
	(0)		5 mg/kg/day (730)								
PCB	(I)										
	(0)										
HCB	(I)										
	(0)				50 mg/dog/day (21)	100 mg/dog/day (365)		50 mg/dog/day (21)			

[a] All values are the lowest dose level reported for each listed effect category, from the mammalian toxicity sections of Chapters 7-12, with dosing duration listed in (days). A blank indicates that the effect has not been reported in this species for this isomer.

[b] From subchronic, chronic, reproductive and teratogenicity studies

[c] mg/m^3, 6 hours/day, 5 days/week

[d] mg/m^3, 7 hours/day, 5 days/week

I = Inhalation exposure; 0 = oral exposure

Mono-CB = monochlorobenzene; DCB = dichlorobenzene; TCB = trichlorobenzene; TeCB = tetrachlorobenzene; PCB = pentachlorobenzene; HCB = hexachlorobenzene

TABLE 13-12

Comparison[a,b] of Toxic Effects of Chlorinated Benzenes in Monkeys

Chemical		Organ/Body Weight Changes	Altered Enzyme Levels	Porphyrinogenic Effects	Neurologic Effects	Hematopoietic Effects	Renal Effects	Hepatic Effects	Adrenal Effects	Reproductive and Teratogenic Effects	Carcinogenic Effects
Mono-CB	(I)										
	(O)										
1,2-DCB	(I)										
	(O)										
1,3-DCB	(I)										
	(O)										
1,4-DCB	(I)										
	(O)										
1,2,3-TCB	(I)										
	(O)										
1,2,4-TCB	(I)										
	(O)		90 mg/kg/ day (30)		174 mg/kg/ day (30)			174 mg/kg/ day (30)			
1,3,5-TCB	(I)										
	(O)										
1,2,3,4-TeCB	(I)										
	(O)										
1,2,3,5-TeCB	(I)										
	(O)										
1,2,4,5-TeCB	(I)										
	(O)										
PCB	(I)										
	(O)										
HCB	(I)										
	(O)						8 mg/kg/ day (60)	8 mg/kg/day (60)			

[a] All values are the lowest dose level reported for each listed effect category, from the mammalian toxicity sections of Chapters 7-12, with dosing duration listed in (days). A blank indicates that the effect has not been reported in this species for this isomer.

[b] From subchronic, chronic, reproductive and teratogenicity studies

I = Inhalation exposure; O = oral exposure

Mono-CB = monochlorobenzene; DCB = dichlorobenzene; TCB = trichlorobenzene; TeCB = tetrachlorobenzene; PCB = pentachlorobenzene; HCB = hexachlorobenzene

because the variety of studies used to glean the effects information were as follows: the studies were conducted under a wide range of experimental conditions; employed different study durations; used a variety of animal strains and different group sizes; and designed to assess different endpoints. Nevertheless, these tables do allow for a broad comparison of the toxic effects induced by the 12 different chlorinated benzenes in a variety of species.

After reviewing Tables 3-8 through 13-12, it can be seen that large data gaps exist for many of the chlorinated benzenes, especially for 1,3-dichlorobenzene, the trichlorobenzenes and the tetrachlorobenzenes. Also, except for the rat and possibly the mouse, the effects from subchronic and chronic exposure to the different chlorinated benzenes in a variety of animal species have not been studied. The interpretation of possible chlorinated benzenes structure activity relationships will, for the most part, be left to the document reader. The only interpretation that will be proposed from these comparison tables is an apparent trend, for many of the toxic effect categories, of increased toxicity with increased chlorination of the benzene ring.

13.2.2. **Estimated Toxicity Thresholds.** Estimated toxicity threshold levels as determined from the studies discussed in the respective mammalian toxicity sections of Chapters 7-12 of this document are presented in Table 13-13.

13.3. CARCINOGENICITY STUDIES

Adequate evidence of the carcinogenicity of the different chlorinated benzenes has only been found for hexachlorobenzene. The other chlorinated benzenes either have not been studied for their carcinogenicity or the studies that have been conducted are inadequate.

TABLE 13-13

Toxicity Data for Threshold Estimates

Compound	Species	Route	Dose Concentration	Dose Duration	Effect Level	Reference
Monochlorobenzene	dog	inhalation	0.75 mg/ℓ (162 ppm), 6 hour/day, 5 day/week	62 exposures over 90 days	NOEL^a	Monsanto, 1978
Monochlorobenzene	rat	inhalation	2.0 mg/ℓ, 6 hour/day, 5 day/week	62 exposures over 90 days	NOEL^a	Monsanto, 1978
Monochlorobenzene	dog	oral	27.3 mg/kg/day	90 days	NOEL^a	Monsanto, 1967a
Monochlorobenzene	rat	oral	50 mg/kg/day	93-99 days	NOEL^a	Monsanto, 1967b
Monochlorobenzene	rat	oral	14.4 mg/kg/day	192 days	NOAEL^b	Irish, 1963
Monochlorobenzene	rat	oral	125 mg/kg/day, 5 day/week	13 weeks	NOEL^a	NTP, 1983
Monochlorobenzene	rat	oral	250 mg/kg/day, 5 day/week	13 weeks	LOAEL^a	NTP, 1983
Monochlorobenzene	mouse	oral	60 mg/kg/day, 5 day/week	13 weeks	LOAEL^a	NTP, 1983
1,2-Dichlorobenzne	rat, rabbit, monkey	inhalation	560 mg/m^3, 7 hour/day, 5 day/week	6-7 months	NOEL^a	Hollingsworth et al., 1958
1,2-Dichlorobenzene	guinea pig	inhalation	290 mg/m^3, 7 hour/day, 5 day/week	6.5 months	NOEL^a	Hollingsworth et al., 1958
1,2-Dichlorobenzene	rat	oral	18.8 mg/kg, 5 day/week	138 doses	NOEL^a	Hollingsworth et al., 1958
1,2-Dichlorobenzene	rat	oral	0.001 mg/kg/day	5 months	NOEL^a	Varshavskaya, 1967a
1,2-Dichlorobenzene	rat	oral	30 mg/kg, 5 day/week	13 weeks	LOAEL^a	NTP, 1982
1,2-Dichlorobenzene	mouse (female)	oral	250 mg/kg, 5 day/week	13 weeks	NOEL^a	NTP, 1982
1,2-Dichlorobenzene	mouse (male)	oral	125 mg/kg, 5 day/week	13 weeks	NOEL^a	NTP, 1982
1,4-Dichlorobenzene	rat, guinea pig, mouse, rabbit, monkey	inhalation	580 mg/m^3, 7 hour/day, 5 day/week	6-7 months	NOEL^a	Hollingsworth et al., 1956
1,4-Dichlorobenzene	rat	oral	18.8 mg/kg, 5 day/week	138 doses	NOEL^a	Hollingsworth et al., 1956

TABLE 13-13 (cont.)

Compound	Species	Route	Dose Concentration	Dose Duration	Effect Level	Reference
1,2,4-Trichlorobenzene	rat	inhalation	22.3 mg/m^3, 6 hour/day, 5 day/week	3 months	NOAEL[a]	Watanabe et al., 1978
1,2,4-Trichlorobenzene	rabbit, monkey	inhalation	742 mg/m^3, 7 hour/day, 5 day/week	26 weeks	NOEL[a]	Coate et al., 1977
1,2,4-Trichlorobenzene	monkey	oral	25 mg/kg/day	30 days	NOEL[a]	Smith et al., 1978
1,3,5-Trichlorobenzene	rat	inhalation	74.2 mg/m^3, 6 hour/day, 5 day/week	13 weeks	NOAEL[a]	Sasmore and Palmer, 1981
1,2,4,5-Tetrachlorobenzene	rat, rabbit	oral	0.001 mg/kg/day	8 months	NOEL[a]	Fomenko, 1965
Pentachlorobenzene	rat	oral	250 mg/kg diet (~16-31 mg/kg/day)	180 days	NOEL[a]	Linder et al., 1980
Pentachlorobenzene	rat	oral	500 mg/kg diet (~27-63 mg/kg/day)	180 days	LOAEL[a]	Linder et al., 1980
Pentachlorobenzene	rat (offspring)	oral	125 mg/kg diet (~14-16 mg/kg/day)	gestation and suckling	NOEL[a]	Linder et al., 1980
Pentachlorobenzene	rat (offspring)	oral	50 mg/kg/day	days 6-15 of gestation	LOAEL[a]	Khera and Villeneuve, 1975
Hexachlorobenzene	rat	oral	0.5 mg/kg/day	15 weeks	NOAEL[a]	Kuiper-Goodman et al., 1977
Hexachlorobenzene	rat	oral	2.0 mg/kg/day	15 weeks	LOAEL[a]	Kuiper-Goodman et al., 1977
Hexachlorobenzene	rat	oral	0.32 mg/kg diet (0.01-0.04 mg/kg/day)	gestation-lifetime	NOEL[a]	Arnold et al., 1983
Hexachlorobenzene	rat	oral	20 mg/kg diet	F_0 to F_4 generations	NOEL[a]	Grant et al., 1977

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[a] Estimated toxicity thresholds as determined in the respective Mammalian Toxicity Sections of this document.

[b] Estimated toxicity thresholds as found in U.S. EPA, 1980b.

NOEL - No-observed-effect level: That exposure level at which there are no statistically significant increases in frequency or severity of effects between the exposed population and its appropriate control.

NOAEL = No-observed-adverse-effect level: That exposure level at which there are no statistically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control. Effects are produced at this dose, but they are not considered to be adverse.

LOAEL - Lowest-observed-adverse-effect level: The lowest exposure level in a study or group of studies which produces statistically significant increases in frequency or severity of effects between the exposed population and its appropriate control.

The chlorinated benzenes for which animal carcinogenicity studies were available for review were hexachlorobenzene, 1,2,4-trichlorobenzene, 1,2-dichlorobenzene and monochlorobenzene. One study which included both rats and mice, was available for monochlorobenzene and for 1,2-dichlorobenzene. The chronic studies which were available for hexachlorobenzene included two on hamsters, one on mice and four on rats as well as a few studies which failed to qualify as carcinogenicity tests.

All of the hexachlorobenzene carcinogenicity studies, with the exception of one, conducted at low doses, yielded one or more sites of significantly increased tumor incidence. The primary target organ appears to be the liver, but thyroid, parathyroid and adrenal glands also showed increases and in one instance kidney tumors were increased.

Malignant liver tumors occurred in one experiment on hamsters (Cabral et al., 1977) where the incidence of haemangioendothelioma was 6/30 (20%) in treated males at 8 mg/kg bw/day compared with 0/40 (0%) in controls and an incidence of 7/60 (12%) in treated females at 16 mg/kg bw/day compared with 0/39 (0%) in controls. In an experiment in rats Lambrecht (1983) obtained hepatocellular carcinoma in hexachlorobenzene-treated males with a frequency of 3/52 (6%) compared to 0/54 (0%) in controls and of 36/56 (64%) in treated females compared to 0/52 (0%) in controls. These effects were produced at 4-5 mg/kg bw/day.

Hepatoma was increased as a result of hexachlorobenzene in two hamster studies (Cabral et al., 1977; Lambrecht et al., 1982). In the latter report the number of animals at risk was small and the increase just detectable. In the Cabral study at a dose of 4 mg/kg bw/day both males and females had a 14/30 (47%) incidence of hepatoma while controls for each sex had 0/40 (0%). The incidence went as high as 51/60 (85%) at the largest dose used.

Cabral (1979) also found an incidence of 3/12 (25%) in both male and female mice administered 6 mg/kg bw/day hexachlorobenzene compared with 0/50 (0%) in controls. The rat studies in which hepatomas were reported are those of Smith and Cabral (1980) and Lambrecht et al. (1983). In the study of Smith and Cabral (1980) an Agus rat strain was used that is particularly susceptible to porphyria and liver tumors. These animals, all female, gave 100% yield of hepatoma in 14 animals compared to 0/12 (0%) in controls. Part of that same study employed six female Wistar rats in a treated group and 4 of 6 cases of hepatoma (67%) were reported compared with 0/4 (0%) in controls. In Lambrecht's study at 4-5 mg/kg bw/day Sprague-Dawley rats developed hepatoma in 19% of treated males and 46% of treated females while controls for both sexes were 0/52 (0%). They also found hepatocellular carcinoma in females with an incidence of 36/56 (64%) at 4-5 mg/kg/day and 48/55 (87%) at 8-10 mg/kg/day.

The liver carcinogenicity and tumorigenicity of hexachlorobenzene, therefore, seems established by repeated experiments in rats and hamsters and by a single study in mice. A high incidence is induced with doses as low as 4-5 mg/kg bw/day. This dosage appears to be effective in three rodent species in inducing hepatoma. In the Lambrecht (1983) study the oral dose was nominally 4-5 mg/kg bw/day in the diet, but may have been higher because of inhalation exposure from hexachlorobenzene in the feed, since the compound was not solubilized in any liquid vehicle.

Other tumors were reported as well as those which occurred in the liver. In male hamsters, thyroid tumors were significantly elevated at 16 mg/kg bw/day in males and in a 2-generation study, rats of the F_1 generation had significant increase in adrenal pheochromocytoma in females and parathyroid tumors in males. These tumors may not be spurious for the following reasons. The doses involved did not produce significant toxicity and it is unlikely that nonspecific stress or systemic toxicity evoked these responses. Also, one of the observations made on humans accidentally exposed to hexachlorobenzene, initially and in a 25-year follow-up, is thyroid enlargement well above expected levels for that area (Peters et al., 1982). In addition, rats exposed to monochlorobenzene had a significant decrease in pituitary adenoma incidence suggesting that the endocrine balance may be affected by chlorinated benzenes.

There was one report of a significant increase in renal cell adenoma in rats of both sexes at 4-5 mg/kg bw/day.

The studies on 1,2-dichlorobenzene and monochlorobenzene were conducted at doses which were well below the MTD as estimated by subchronic range finding studies. In the case of 1,2-dichlorobenzene in rats no increase in tumors or other pathology was found. In mice no tumor type was significantly increased compared with controls.

In the case of the monochlorobenzene a significantly increased incidence of neoplastic nodules in male rats was induced at a gavage dose of 120 mg/kg bw/day. The data on the 1,2-dichlorobenzene and monochlorobenzene are inadequate to draw conclusions concerning the human carcinogenicity of these compounds.

For hexachlorobenzene the studies showing positive tumor responses are summarized in Table 13-14. It has induced liver tumors in hamsters, mice and rats, thyroid tumors in hamsters, and kidney and adrenal tumors in rats. Using the IARC ranking system for classifying the evidence of carcinogenicity, hexachlorobenzene would be a Group 2 chemical which IARC describes as a probable carcinogen in humans.

TABLE 13-14

Summary of Tumors Induced in Rodents by HCB

Species	Lowest to Produce Tumor mg/kg bw/day	Males		Females		Reference
		Tumor Type	% Treated/Control	Tumor Type	% Treated/Control	
Hamsters	4	hepatoma	47/0	hepatoma	47/0	Cabral, 1977
Hamsters	8 male; 16 female	haemangioendothelioma of liver	20/0	haemangioendothelioma of liver	12/0	Cabral, 1977
Hamsters	16	thyroid adenoma	14/0	thyroid adenoma	6/0	Cabral, 1977
Hamsters	200 ppm	hepatoma	8/0	hepatoma	8/0	Lambrecht et al., 1982a
Mice	6	hepatoma	25/0	hepatoma	25/0	Cabral, 1979
Rats	6-8			hepatoma (Agus)	100/0	Smith and Cabral, 1980
Rats	6-8			hepatoma (Wistar)	67/0	Smith and Cabral, 1980
Rats	F_1 dose unknown in utero, adult = 0.4	parathyroid	25/4			Arnold, 1983
Rats	F_1 dose unknown in utero, adult = 0.4	adrenal pheochromocytoma	35/23	adrenal pheochromocytoma	35/4	Arnold, 1983
Rats	Vit. A content varied, HCB = 0.4	none		none		Arnold, 1983

TABLE 13-14 (cont.)

Species	Lowest to Produce Tumor mg/kg bw/day	Males		Females		Reference
		Tumor Type	% Treated/Control	Tumor Type	% Treated/Control	
Rats	4-5	hepatoma	19/0	hepatoma	46/0	Lambrecht, 1983
Rats	4-5	hepatocellular carcinoma	6/0	hepatocellular carcinoma	64/0	Lambrecht, 1983
Rats	4-5	renal cell adenoma	79/13	renal cell adenoma	13/2	Lambrecht, 1983

A quantitative estimate of the carcinogenic potency of hexachlorobenzene and an upper-bound estimate of the risks from continuous human exposure to 1 μg/m³ in air and 1 μg/ℓ in drinking water were made from data on the hepatocellular carcinoma response in female rats. The upper-bound slope of the dose-response curve, q_1^*, is 1.7/(mg/kg/day), giving a potency index which is in the second quartile of 54 suspect carcinogens evaluated by the Carcinogen Assessment Group. The unit risks for air and water exposures are 4.9×10^{-4} for 1 μg/m³ in ambient air and 4.9×10^{-5} for 1 μg/ℓ in drinking water. Corresponding estimates from 13 other data sets, encompassing different tumor sites and animal species, fall within a factor of 10 of these estimates except for thyroid tumors in hamsters, which give estimates of about 1/20 of the potency based on the rat hepatocellular carcinoma response.

13.4. HUMAN STUDIES

Although animal studies indicate that hexachlorobenzene is carcinogenic in hamsters, rats and mice, there were no adequate epidemiologic studies available to corroborate these findings in humans. However, the human data which has been collected were not designed to detect human carcinogenicity, but rather provide a better understanding of hexachlorobenzene toxicity in infants (pink sore) and adults (porphyria cutanea tarda) (Cam, 1963; Cripps et al., 1981; Peters et al., 1966; Peters et al., 1982). In the studies of hexachlorobenzene-induced toxicity, human consumption of hexachlorobenzene through contaminated wheat was estimated at 50-200 mg hexachlorobenzene/person/day (0.71-2.86 mg/kg bw/day for a 70 kg male); these doses were sufficient to cause porphyria cutanea tarda and other effects in 3000-5000 people (Courtney, 1979). Epidemiologic studies with occupationally-exposed workers or people living in the vicinity of a chlorinated solvents plant were not designed to detect carcinogenicity. The exposure information provided by those studies is not sufficient to relate dose level to effect (Currier et al., 1980).

Two other chlorinated benzenes were reported to have effects in humans. 1,2-Dichlorobenzene (Zapata-Gayon et al., 1982) and 1,2,4,5-tetrachlorobenzene (Kiraly et al., 1979) each caused statistically significant increases in the frequency of chromosomal aberrations, but neither study reported the ambient atmospheric concentration. Thus, these two chlorobenzenes are clastogenic, but the critical exposure concentration is not known.

13.5. FACTORS INFLUENCING HEALTH HAZARD ASSESSMENT

13.5.1. Exposure. For an individual or a population, exposure to potentially toxic substances occurs on two levels. The first is exposure to ambient environmental levels which occurs through food, drinking water and air. Physiologic exposure is the second and more important level and occurs after the compound has been absorbed and is in a position to interact directly with critical cellular components. This interaction is the basis for toxicologic effects.

Chlorinated benzenes in the environment are resistant to biotransformation and degradation and are, therefore, ecologically persistent compounds. At the level of the individual organism, these compounds are biologically persistent because of their affinity for fatty tissues and their slow rate of biotransformation or elimination (see Section 5.3.). Thus, biological persistence and bioaccumulation in nonhuman organisms increase the likelihood of human exposure. Tables 13-15 and 13-16 present some useful properties and trends of chlorinated benzenes which illustrate the differences that exist between the chlorinated benzenes isomers and their potential for human exposure.

TABLE 13-15

Comparison of Chemical and Physical Properties of Chlorinated Benzenes

Chemical	Molecular Weight[a]	Melting Point (°C)[a]	Boiling Point (°C)[a]	Density g/mc (20°C)[a]	Log P*[a]	Volatility in Vapor Pressure MM Hg at 25°C[b]	
Monochlorobenzene	112.56	-45.6	132	1.1058	2.84	11.8	Likely to be present as vapor in ambient air
Dichlorobenzene							
1,2-	147.01	-17.0	180.5	1.3048	3.38	1.28	
1,3-	147.01	-24.7	173	1.2828 (25)	3.38	1.89	
1,4-	147.01	53.1	174	1.2475	3.39	1.0	
Trichlorobenzene							
1,2,3-	181.46	52.6	221	1.69	4.1	0.07	
1,2,4-	181.46	16.95	213.5	1.4542	4.12	0.29	
1,3,5-	181.46	63.4	208.4	1.3865 (64)	NA	0.15	
Tetrachlorobenzene							
1,2,3,4-	215.90	47.5	254	NA	NA	0.04	Not likely to be present in ambient air -- more likely to be present in condensed state in soil etc.
1,2,3,5-	215.90	54.5	246	NA	NA	0.07	
1,2,4,5-	215.90	139.5	246	1.858 (22)	4.93	0.05	
Pentachlorobenzene	250.34	86	277	1.8342 (16.5)	5.63	~0	
Hexachlorobenzene	284.76	230	3229	1.569 (23)	5.8	1.68×10^{-5}	

[a] Increasing trend

[b] Decreasing trend

NA = Not available

P* = Partition coefficient at 25°C

TABLE 13-16

Comparison of Chlorinated Benzenes BCF and Water Concentrations

Chemical	BCF[a] (rainbow trout)	Mean Chlorobenzene Concentrations in Drinking Water[b] (ng/ℓ)	Chlorinated Benzenes in Various Wastewaters Mean Concentration (μg/ℓ)
Monochlorobenzene	46	NA	667
Dichlorobenzene			
1,2-	270-560	3	141
1,3-	420-740	1	21
1,4-	370-720	13	79
Trichlorobenzene			
1,2,3-	1,200-2,600	0.1	NA
1,2,4-	890-3,200	2	161
1,3,5-	1,800-4,100	<0.1	NA
Tetrachlorobenzene			
1,2,3,4-	5,200-12,000	0.3	NA
1,2,3,5-	NA	<0.05	NA
1,2,4,5-	5,300-13,000	0.2	NA
Pentachlorobenzene	13,000-20,000	0.04	NA
Hexachlorobenzene	5,500-20,000	0.1	NA

[a] Increasing trend

[b] Decreasing trend

BCF = Bioconcentration factor; NA = not available

Although toxic effects in humans have not been directly related to ambient chlorinated benzene exposure, it is apparent from the residue levels in human tissues that humans receive physiologic exposures to the chlorinated benzenes (see Section 4.3.5.). A comparison of human ambient exposure levels and tissue concentrations confirms that humans bioaccumulate chlorinated benzenes (Burn et al., 1974; Currier et al., 1980). Prolonged physiologic exposure and the uncertainty of the toxic effects of chronic low-dose exposure to the chlorinated benzenes increase the concern for human exposures resulting from ambient levels of these substances.

The large number of locations at which chlorinated benzenes have been detected indicate their ubiquity in the environment and is a reflection of their annual production volume, release rate, end uses (Sections 4.1.-4.3.), and their environmental transport and fate (Sections 5.1.-6.3.). Human exposure to these ambient concentrations depends on the chlorobenzene concentration in, and absorption efficiency from, air, drinking water or food. The relative contribution of each medium to the total human exposure was estimated from monitoring data for several areas of the United States; the limitations of these estimates are discussed in Section 4.4. The estimated yearly exposures to the chlorinated benzenes from air are shown in Table 13-17 and are based on the data for each chlorobenzene shown in Table 4-8. The available data indicate that human exposure to chlorinated benzenes through inhalation may be greater than ingestion exposure either through drinking water or through foods. The relative contribution of food to human exposure is less certain because food has not been extensively monitored for chlorinated benzene residues; two studies estimated annual hexachlorobenzene exposures of 0.026 mg/year and 0.145 mg/year, respectively (IARC, 1979).

TABLE 13-17

Estimated Yearly Exposure to Several Chlorinated Benzenes Via Inhalation

Chemical	Mean Ambient Concentration (ng/m^3)*	Exposure (mg/yr) Adult Man	Adult Woman	Child (10 yr)	Infant (1 yr)
Monochlorobenzenes	3087	25.9	23.8	17.0	4.3
1,2-Dichlorobenzene	1142	9.6	8.8	6.3	1.6
1,3-Dichlorobenzene	571	4.8	4.4	3.1	0.8
1,4-Dichlorobenzene	1563	13.1	12.0	8.6	2.2
Trichlorobenzenes	136	1.1	1.0	0.7	0.2
Tetrachlorobenzenes	3502	29.4	27.0	19.3	4.9

*Mean levels obtained from Table 4-8

This paucity of data for food, however, does not preclude this medium as a significant human exposure route. Trout from the Great Lakes, for example, had detectable levels of all of the chlorobenzenes except monochlorobenzene (Oliver and Nicol, 1982), and it is conceivable that other animals used as food sources also have tissue residues of chlorobenzenes.

13.6. REGULATIONS AND STANDARDS

The chlorinated benzenes are regulated under numerous United States and foreign statutes. These have been grouped according to the type of activity or medium being controlled.

13.6.1. Occupational Standards.

13.6.1.1. **MONOCHLOROBENZENE** -- The current OSHA standard for monochlorobenzene levels in the workplace is 75 ppm (350 mg/m^3). This threshold limit value (TLV), established in 1974, is not to be exceeded for an 8-hour time weighted average (TWA) for an employee's exposure in any 8-hour shift of a 40-hour workweek (29 CFR 1910). This standard is identical to those recommended by the American Conference of Governmental Industrial Hygienists (ACGIH, 1982). Occupational standards for monochlorobenzene have also been established in four foreign countries. These are presented in Table 13-18.

The Interagency Testing Committee (ITC) designated monochlorobenzene a TSCA Section 4(e) priority chemical in its Initial Report to the Administrator of EPA (44 FR 70666). Additionally, all manufacturers and producers of monochlorobenzene were required to report exposure, production and use information to EPA's Office of Toxic Substances in the form of a Preliminary Assessment Information Manufacturers Report. The deadline for submission was November 19, 1982 (40 CFR 712).

TABLE 13-18

Occupational Standards for Monochlorobenzene*

Country	TLV ppm	TLV mg/m^3	Year Adopted
USSR	11	50	1972
German Democratic Republic	--	50	1973
Czechoslovakia	--	200	1969
Federal Republic of Germany	50	230	1974
USA	75	350	1974

*Source: Verschueren, 1977

13.6.1.2. **DICHLOROBENZENES** -- The OSHA standard for 1,2-dichlorobenzene in the workplace is set at a ceiling of 50 ppm (300 mg/m^3). Levels in the workplace are at no time permitted to exceed this value (39 FR, No. 125). The 1982 ACGIH TLV for 1,2-dichlorobenzene is identical (ACGIH, 1982). Foreign standards for occupational exposure to 1,2-dichlorobenzene are shown in Table 13-19.

In 1978, NIOSH classified 1,2-dichlorobenzene as a Group II pesticide (a pesticide that poses "adverse acute health risks at moderate doses") and recommended criteria for standards for occupations in pesticide manufacturing and formulating (NIOSH, 1978). These standards rely on engineering controls, work practices and medical surveillance programs, rather than workplace air limits, to protect workers from adverse effects of pesticide exposure in pesticide manufacturing and formulating. NIOSH specifically chose not to establish scientifically valid environmental (workplace air) limits for pesticides (except those already promulgated) because exposure via other routes, especially dermal, had proven to be of critical importance for many pesticides and NIOSH believed that "immediate action" was needed to protect workers in pesticide manufacturing and formulating plants (NIOSH, 1978).

The current OSHA standard for 1,4-dichlorobenzene in the workplace is a TLV of 75 ppm, 450 mg/m^3 (39 FR, No. 125). In addition to recommending a TLV identical to the OSHA standard, ACGIH has recommended a short-term exposure limit (STEL), the maximum concentration allowable in a 15-minute period, of 110 ppm (675 mg/m^3) for 1,4-dichlorobenzene (ACGIH, 1982). NIOSH has also classifed 1,4-dichlorobenzene as a Group II pesticide and recommended criteria for workplace standards in pesticide manufacturing and formulating plants (NIOSH, 1978). Foreign standards for occupational exposure to 1,4-dichlorobenzene are presented in Table 13-20.

TABLE 13-19

Occupational Standards for 1,2-Dichlorobenzene[a]

Country (Standard)	Level		Year Adopted
	ppm	mg/m3	
USSR (TLV)[b]	3	20	1972
German Democratic Republic (TLV)	--	150	1973
USA (MAC)[c]	50	300	1974
Federal Republic of Germany (TLV)	50	300	1974

[a] Source: Verschueren, 1977

[b] Threshold limit value

[c] Maximum allowable concentration

TABLE 13-20

Occupational Standards for 1,4-Dichlorobenzene*

Country	TLV ppm	TLV mg/m^3	Year Adopted
USSR	--	20	1972
German Democratic Republic	--	200	1973
USA	75	450	1974
Federal Republic of Germany	75	450	1974

*Source: Verschueren, 1977

There are no occupational workplace standards, either United States or foreign, for 1,3-dichlorobenzene. However, dichlorobenzenes (no isomer specified) were designated by the ITC as TSCA Section 4(e) priority chemicals (44 FR 70666). Separate Preliminary Assessment Information Manufacturers Reports on 1,2-, 1,3- and 1,4-dichlorobenzene were to be submitted to EPA by November 19, 1982 (40 CFR 712).

13.6.1.3. TRICHLOROBENZENES -- There are no United States workplace standards for the trichlorobenzenes.

The ACGIH has recommended a ceiling of 5 ppm (40 mg/m^3) for 1,2,4-trichlorobenzene (ACGIH, 1982), and NIOSH classified it as a Group III pesticide. Group III pesticides are less toxic than Group II pesticides and the recommended criteria for workplace standards are less stringent than those recommended for Group II pesticides (NIOSH, 1978). The British Journal of Industrial Medicine reported a provisional operational limit of 25 ppm for 1,2,4-trichlorobenzene (Verschueren, 1977). The 1971 TLV for 1,2,3-trichlorobenzene is 1.3 ppm [10 mg/m^3 (n.s.i.)] for the USSR (Verschueren, 1977).

Trichlorobenzenes have been designated by the ITC as TSCA Section 4(e) priority chemicals (44 FR 70666). Preliminary Assessment Information Manufacturers Reports were to be submitted to the EPA Office of Toxic Substances by November 19, 1982, for each of the trichlorobenzenes (40 CFR 712).

13.6.1.4. TETRACHLOROBENZENES AND PENTACHLOROBENZENE -- There are no occupational workplace standards or recommended criteria for standards, United States or foreign, for the tetrachlorobenzenes or pentachlorobenzene. These chlorobenzenes have been designated as TSCA Section 4(e) priority chemicals (44 FR 70666). Preliminary Assessment Information Manufacturers Reports were required on 1,2,3,4-tetra-, 1,2,3,5-tetra- and 1,2,4,5-tetrachlorobenzene and pentachlorobenzene (40 CFR 712).

13.6.1.5. HEXACHLOROBENZENE -- Workplace standards have not been established in the United States. The USSR has established a TLV of 0.08 ppm (0.9 mg/m^3) (Verschueren, 1977). NIOSH classified hexachlorobenzene as a Group II pesticide and recommended criteria for standards (NIOSH, 1978).

13.6.2. Transportation Regulations. The Department of Transportation (DOT), the Coast Guard and the Departments of Commerce and Energy regulate, in varying degrees, the transport of the chlorinated benzenes.

All of the chlorinated benzenes are regulated under the Hazardous Material Transportation Act (HMTA) as amended by the Comprehensive Environmental Response Compensation and Liability Act (CERCLA), i.e., "Superfund" Act (49 CFR 172.101, 46 FR 17738). The HMTA, administered by DOT, specifies the requirements to be observed in the preparation for interstate shipment and transport of hazardous materials (46 CFR 171-179). CERCLA further classified the chlorinated benzenes as hazardous substances and provides that common carriers of hazardous substances may be held liable for releases of hazardous substances in amounts equal to or greater than the reportable quantity (RQ). The RQs for mono-, 1,2-di- or 1,4-dichlorobenzene are set at 100 pounds (45.4 kg) (49 CFR 172.101). RQs for the remaining chlorinated benzenes have been set at 1 pound pending establishment of different RQs by EPA (46 CFR 17738).

DOT has designated monochlorobenzene as a flammable liquid. The maximum net quantity permitted in one package for transport by passenger carrying aircraft or railcar has been set at 1 quart, while the maximum net quantity for cargo aircraft has been set at 10 gallons/package (49 CFR 172.101).

The U.S. Coast Guard regulates the transport of hazardous materials while aboard vessels. Title 46, Part 150, specifies the compatibility of cargoes and operating requirements for bulk liquid hazardous waste cargoes;

i.e., monochlorobenzene, dichlorobenzene (no isomer specified) and 1,2,4-trichlorobenzene (46 CFR 150). Part 153 prescribes the safe handling procedures for self-propelled vessels carrying hazardous liquids; i.e., mono- and dichlorobenzene (no isomer specified) (46 CFR 153). Part 151 details the minimum requirements for unmanned tank barges carrying bulk dangerous cargoes; i.e., monochlorobenzene (46 CFR 151).

The international transport of hazardous materials is regulated by the International Maritime Dangerous Goods Code (IMCO) and administered by DOT in this country. Mono-, 1,2-di- and 1,4-dichlorobenzene are regulated under the IMCO code (46 FR 29392, 49 CFR 172.102).

The export of the chlorobenzenes (i.e., mono-, 1,2-di-, 1,4-di, 1,2,3-tri-, 1,2,4-tri- and hexachlorobenzene) is regulated by the DOT via the departments use of the Commodity Control List (15 CFR 399). The DOE regulates the import of oil and petrochemicals, and mono-, di- and tetrachlorobenzene (no isomers specified) are classified as petrochemicals under the authority of the Oil Import Regulations (10 CFR 213).

13.6.3. Solid Waste Regulations. Under the Solid Waste Disposal Act as amended by the Resources Conservation and Recovery Act (RCRA), EPA has designated mono-, 1,2-di-, 1,3-di-, 1,4-di-, 1,2,4,5-tetra-, penta- and hexachlorobenzene as hazardous wastes (40 CFR 261.33); subject to the disposal and permit regulations of Title 40 Code of Federal Regulations, Parts 262-265 and Parts 122-124 (40 CFR 261). All of the chlorinated benzenes are designated as hazardous constituents of hazardous wastes from specific sources subject to RCRA disposal regulations (40 CFR 261.32). Table 13-21 shows these specific wastes in relation to the chlorinated benzenes. Monochlorobenzene and 1,2-dichlorobenzene, as spent halogenated solvents, and their still bottoms from the recovery of these solvents, are

TABLE 13-21

The Chlorinated Benzenes as Constituents of Hazardous Wastes from Specific Sources*

EPA Hazardous Waste No.	Hazardous Waste	Hazard Constituent
K015	Still bottoms from the distillation of benzyl chloride	Monochlorobenzene
K016	Heavy ends or distillation residues from the production of carbon tetrachloride	Hexachlorobenzene
K018	Heavy ends from the fractionation column in ethyl chloride production	Hexachlorobenzene
K030	Column bottoms or heavy ends from the combined production of trichloroethylene and perchloroethylene	Hexachlorobenzene
K042	Heavy ends or distillation residues from the distillations of tetrachlorobenzene in the production of the 2,3,5- isomer	1,2-Dichloro- and hexachlorobenzene
K085	Distillation or fractionation column bottoms from the production of chlorobenzene	Dichlorobenzenes, trichlorobenzenes, tetrachlorobenzenes, pentachlorobenzene, hexachlorobenzene
K105	Separated aqueous stream from the reactor product washing step in the production of chlorobenzene	Monochlorobenzene, dichlorobenzenes

*Source: 40 CFR 261.32

also regulated for disposal under RCRA (Hazardous Waste No. F002) provided that the combined concentrations of the spent solvent in the resulting mixture is no greater than 25 ppm (46 FR 56582, 40 CFR 261.31).

13.6.4. Food Tolerances. Food tolerances have been established for monochlorobenzene and hexachlorobenzene.

13.6.4.1. **MONOCHLOROBENZENE** -- Monochlorobenzene is exempted from the requirement of a tolerance when used in accordance with good agricultural practices as an ingredient in pesticide formulation applied to growing crops only. Permitted uses are as a solvent or cosolvent if monochlorobenzene contains not more than 1% impurities. Under FIFRA, use of monochlorobenzene is prohibited after edible parts of plants begin to form. The grazing of livestock in treated areas is prohibited within 48 hours after application [40 CFR 180.1001(d)].

The FDA permits the use of polysulfone resins and polycarbonate resins as articles or components of articles for use in producing or holding food. These resins are permitted to contain 500 ppm of monochlorobenzene as a residual solvent in finished (basic) resin (21 CFR 177.1580, 21 CFR 177.2500). Monochlorobenzene as a component of adhesives used in the packaging of food is also regulated by FDA (21 CFR 175.105).

13.6.4.2. **HEXACHLOROBENZENE** -- USDA regulates the use of hexachlorobenzene as a seed treatment for the control of wheat bunt (smut) under the Federal Seed Act (7 CFR 201).

13.6.5. Water Regulations. Under Section 311(b)(2)(A) of the Federal Water Pollution Control Act, EPA designated monochlorobenzene, dichlorobenzene (no isomer specified), 1,2-di- and 1,4-dichlorobenzene as hazardous substances (40 CFR 116.4) and established an RQ of 100 pounds (45.4 kg) for these chlorinated benzenes (40 CFR 117.3). Discharges equal to or greater than the RQ into or upon United States waters are prohibited unless the discharge is in compliance with applicable permit programs (40 CFR 117.11).

Under the Clean Water Act, Section 307(a), EPA has designated chlorinated benzenes (other than dichlorobenzene) and dichlorobenzenes (all isomers) as toxic pollutants, i.e., priority pollutants (40 CFR 401.15). Effluent limitation guidelines, new source performance standards, and pretreatment standards have been developed or will be developed for the priority pollutants for 21 major industries. Specific definitions for classes and categories are set forth in 40 CFR, Parts 402 through 699.

Under the Clean Water Act, Ambient Water Quality Criteria for chlorinated benzenes have been developed (U.S. EPA, 1980a,b). These are summarized in Tables 13-22 and 13-23. The USSR in 1971 established a drinking water standard for monochlorobenzene of 0.02 mg/ℓ, and an organoleptic limit for 1,2-di- and 1,4-dichlorobenzene has been set at 0.002 mg/ℓ (Verschueren, 1977).

13.6.6. Air Regulations. Ambient air quality standards for the chlorinated benzenes have not been established in the United States. Maximum immission concentration (MIC) and maximum emission concentration (MEC) standards have been established in several European countries for monochlorobenzene, 1,2- and 1,4-dichlorobenzene.

Maximum emmission concentration standards; i.e., ambient air quality standards, are used for calculating the minimum stack heights permitted by law. Dispersion of emitted compounds must be such that the addition of these compounds to ground level concentrations does not result in the ambient air quality standard being exceeded more frequently than the allowed percentage. The MEC is the maximum concentration of a specific pollutant in emitted gases. MECs are derived from ambient air quality standards by taking into account the dispersion phenomena (Verschueren, 1977).

TABLE 13-22

Ambient Water Quality Criteria for Chlorinated Benzenes--Aquatic Life[a]

Aquatic Life	Chlorinated Benzenes[b]	Dichlorobenzenes
Freshwater aquatic life		
Acute toxicity	250 μg/ℓ[c]	1120 μg/ℓ[c]
Chronic toxicity	--[d,e]	763 μg/ℓ[c]
Saltwater aquatic life		
Acute toxicity	160 μg/ℓ[c]	1970 μg/ℓ[c]
Chronic toxicity	129 μg/ℓ[c]	--[d]

[a] Source: U.S. EPA, 1980a,b

[b] Includes all of the chlorinated benzenes except the dichlorobenzenes

[c] Toxicity would occur at lower concentrations among species that are more sensitive than those tested.

[d] No data available

[e] Toxicity occurs at concentrations as low as 50 μg/ℓ for fish species exposed for 7.5 days.

TABLE 13-23

Ambient Water Quality Criteria for the
Chlorinated Benzenes for the Protection of Human Health[a]

Compound	From Toxic Properties Ingested Through:		Based on Available:		From the Potential:
	Water and Contaminated Aquatic Organisms	Contaminated Aquatic Organisms Alone	Toxicity Data	Organoleptic Data	Carcinogenic Effects
Monochlorobenzene			488 μg/ℓ	20 μg/ℓ[b]	
Dichlorobenzenes	400 μg/ℓ	2.6 mg/ℓ			
Trichlorobenzenes	--[c]	--[c]			
1,2,4,5-Tetrachlorobenzene	38 μg/ℓ	48 μg/ℓ			
Pentachlorobenzene	74 μg/ℓ	85 μg/ℓ			
Hexachlorobenzene					0[d]

[a] Source: U.S. EPA, 1980a,b

[b] Organoleptic data as a basis for establishing a water quality criteria have limitations and have no demonstrated relationship to potential adverse human health effects.

[c] Due to insufficient data, a criterion was not derived.

[d] Based on the nonthreshold assumption, however, a zero level may not be attainable at the present time and, therefore, levels that may result in incremental increases of cancer risk of the lifetime were estimated at 10^{-5}, 10^{-6} and 10^{-7}. The corresponding recommended criteria are 7.2 ng/ℓ, 0.72 ng/ℓ and 0.072 ng/ℓ, respectively; if estimates are for consumption of aquatic organisms only, the levels are 7.4 ng/ℓ, 0.74 ng/ℓ and 0.074 ng/ℓ.

13.6.6.1. **MONOCHLOROBENZENE** -- Ambient air quality standards for monochlorobenzene have been established in five countries and are shown in Table 13-24. In addition, MEC limits have been established in the Federal Republic of Germany; should emissions exceed 3 kg/hour, then a concentration of 150 mg/m^3 cannot be exceeded (Verschueren, 1977).

13.6.6.2. **DICHLOROBENZENES** -- Maximum emission concentration limits for 1,2- and 1,4-dichlorobenzene have been established in the Federal Republic of Germany. The MEC limits set were the same as those established for monochlorobenzene: 150 mg/m^3 if emissions are >3 kg/hour (Verschueren, 1977).

TABLE 13-24

Maximum Immission Concentration Standards for Monochlorobenzene*

Country	MIC_s			MIC_1		
	mg/m^3	ppm	Average Time	mg/m^3	ppm	Average Time
USSR	0.100	--	20 min	0.100	--	24 hr
German Democratic Republic	0.3	--	30 min	0.1	--	24 hr
Bulgaria	0.1	0.02	20 min	0.1	0.02	24 hr
Federal Republic of Germany-VDI (Assoc. of German Engineers)	15.0	3.0	30 min	5.0	1.0	30 min
Yugoslavia	0.1	0.02	30 min	0.1	0.02	24 hr

*Source: Verschueren, 1977

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APPENDIX A

Comparison Among Different Extrapolation Models

Four models used for low-dose extrapolation, assuming the independent background, are:

Multistage: $P(d) = 1 - \exp[-(q_1 d + \ldots + q_k d^k)]$

where q_i are non-negative parameters.

Probit: $P(d) = \int_{-\infty}^{A + B \ln(d)} f(x)\, dx$

where f(.) is the standard normal probability density function.

Weibull: $P(d) = 1 - \exp[-bd^k]$

where b and k are non-negative paramters.

One-hit: $P(d) = 1 - \exp[-bd]$

where b is a non-negative parameter.

The maximum likelihood estimates (MLE) of the parameters in the multistage and one-hit models are calculated by means of the program GLOBAL82, which was developed by Howe and Crump (1982). The MLE estimates of the parameters in the probit and Weibull models are calculated by means of the program RISK81, which was developed by Kovar and Krewski (1981).

Table A-1 presents the MLE of parameters in each of the four models.

TABLE A-1

Maximum Likelihood Estimate of the Parameters for Each of the Four Extrapolation Models Based on Hepatocellular Carcinomas in Female Rats* (mg/kg/day)

Basis of Interspecies Extrapolation	Multistage	Probit	Weibull	One-hit
Body weights	$q_1 = 2.20 \times 10^{-1}$	$A = -1.35$	$b = 2.20 \times 10^{-1}$	$b = 2.20 \times 10^{-1}$
	$q_2 = 5.01 \times 10^{-5}$	$B = 1.12$	$k = 1.00$	
Body surface area	$q_1 = 1.35$	$A = 6.70 \times 10^{-1}$	$b = 1.35$	$b = 1.35$
	$q_2 = 1.90 \times 10^{-3}$	$B = 1.12$	$k = 1.00$	

*Source: Lambrecht, 1983